Facultad de Biología. Departamento de Edafología y Química Agrícola. Universidad de Santiago de Compostela

Soil organic matter (SOM) characterisation and biogeochemistry of variable-charge soils

Caracterización de la materia orgánica del suelo (SOM) y biogeoquímica de suelos de carga variable

MANUEL SUÁREZ ABELENDA

Santiago de Compostela, en febrero de 2013

Supervisors:

Marta Camps Arbestain Professor of the Institute of Natural Resources, Massey University, Palmerston North, New Zealand

Felipe Macías Vázquez Professor of the Department of Edafología y Química Agrícola, Biology Faculty, Santiago de Compostela University (USC)

Facultad de Biología. Departamento de Edafología y Química Agrícola. Universidad de Santiago de Compostela

Marta Camps Arbestain, profesora del Institute of Natural Resources, Massey University, Palmerston North, New Zealand y Felipe Macías Vázquez, catedrático del Departamento de Edafología y Química Agrícola, Facultad de Biología de la Universidad de Santiago de Compostela,

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Que la presente memoria, titulada "Soil organic matter (SOM) characterisation and biogeochemistry of variable-charge soils", realizada por el doctorando Manuel Suárez Abelenda para optar al grado de Doctor, reúne todos los requisitos y condiciones necesarias como trabajo de Tesis doctoral.

Y para que conste, dan el visto bueno para su presentación ante la Comisión de Doctorado de la Universidad de Santiago de Compostela.

En Santiago de Compostela, a 18 de febrero de 2013.

Marta Camps Arbestain Felipe Macías Vázquez

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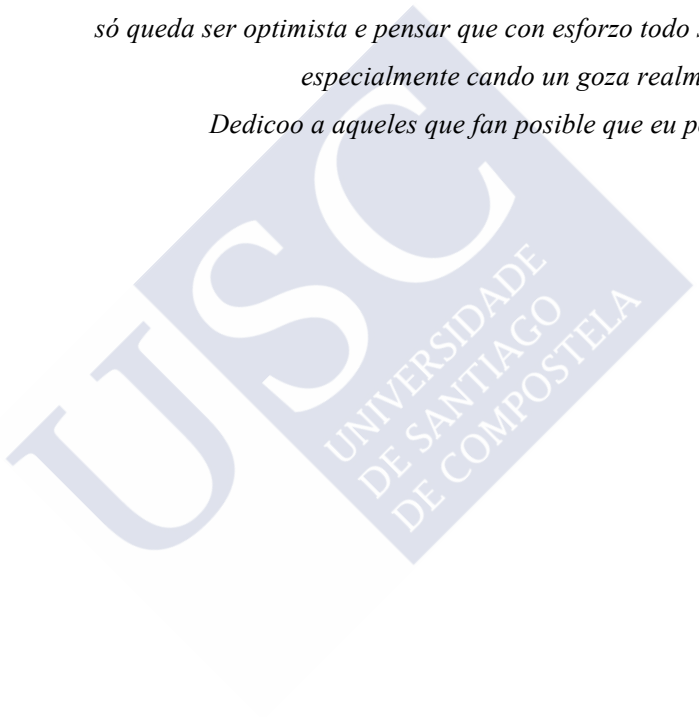
A pesar das dificultades que está atravesando a ciencia nestes últimos anos,
principalmente no noso país, e o futuro incerto que nos depara,
só queda ser optimista e pensar que con esforzo todo se pode acadar,
especialmente cando un goza realmente do que fai.
Dedicoo a aqueles que fan posible que eu poida intentalo...



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...ós meus pais

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List of abbreviations

AVS	acid volatile sulphides; sulfuros ácidos volátiles
BC	black carbon; carbón negro
BN	black carbon derived nitrogen compounds; compuestos nitrogenados derivados del carbón negro
CEC	cation-exchange capacity; capacidad de intercambio catiónico
CP	cross polarisation; polarización cruzada
DOP	degree of pyritization; grado de piritización
DP	direct polarisation; polarización directa
GHG	greenhouse gases; gases de efecto invernadero
Gt	gigatonne; gigatonelada (10^{15} g)
HS	humic substances; sustancias húmicas
MAH	monocylic aromatic hydrocarbon; hidrocarburos aromáticos monocíclicos
non-WAM	non-wastewater affected mangroves; manglares no afectados por aguas residuales
OC	organic carbon; carbón orgánico
PAH	polycylic aromatic hydrocarbon; hidrocarburos aromáticos policíclicos
Py-GC/MS	pyrolysis-gas chromatographic-mass spectroscopy; pirólisis-cromatografía de gases-espectroscopía de gases

solid-state ^{13}C NMR/CPMAS	solid-state ^{13}C cross polarization-magic angle spinning-nuclear magnetic resonance; ^{13}C resonancia magnética nuclear en estado sólido de polarización cruzada con rotación de ángulo mágico
SOM	soil organic matter; materia orgánica del suelo
TIC	total ion current; corriente total de iones
TQPA	total quantified peak area; área total de los picos cuantificados
WAM	wastewater affected mangroves; manglares afectados por aguas residuales

SUMMARY/SUMARIO

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SUMMARY

Environmental frame

GHG (Greenhouse Gases) –such as CO_2, CH_4, N_2O and $H_2O_{(g)}$– emissions have occurred naturally throughout the Earth's history. However during the last 150 y, anthropic activities such as fuel burning, extensive use of agricultural/farming activities, deforestation and/or soil change use have increase GHG emissions, especially those of CO_2 and CH_4. The emissions of such gases to atmosphere are responsible for the so-called "climatic forcing" or "greenhouse effect". Briefly, this phenomenon is produced by the absorbance of solar radiation after bouncing off (reflection) the Earth's surface. As a consequence, the global average temperature is within a range that allows life on Earth. However, during this past century anthropogenic GHG emissions have increased greatly and, according to the IPCC, this has been paralleled by an increase in temperature.

Strategies for the mitigation and adaptation to the current climatic forcing, such as enhanced sequestration of atmospheric CO_2 through photosynthesis of plant biomass, has been endorsed by agencies such as the Intergovernmental Panel on Climate Change (IPCC). However, soils may store two-fold the amount of C stored in the plant biomass and stabilized soil organic C (OC) with longer turnover than plant biomass. Thus, the advancement in the study and the understanding of the mechanisms implied in the soil organic matter (SOM) stabilization is needed so that the OC stock in soils are built up thus mitigating CO_2 emissions.

Soil organic matter and its stabilization

The concept of the SOM structure has notably changed in the last years. The historical view was determined by the presence of a set of partially to highly polycondensed, amorphous, dark-coloured material with wide range molecular weight. New insights suggest a molecular structure composed by relatively simple molecules, whose composition does not necessarily determine its stability, and that the relative persistence of SOM is mainly promoted by complex interactions of both SOM and nearby environment, in particular those with presence of metallic

cations in the soil solution and coloids of high to low degree of crystallization. SOM is not thermodynamically stable under oxic conditions. Its presence in soil is explained to the existence of non-ideal conditions for OC to become oxidised to CO_2. These non-ideal conditions are associated to the lack of the activation energy needed for such reaction. These are favoured by the different SOM protecting mechanisms (e.g., physical and chemical protection) in soil as well determined environmental conditions. All of these halt the activity of microorganisms, the catalizers of these decomposition reactions. The main factors implied in the stabilization mechanisms of SOM in soils are: (i) the intrinsic recalcitrance of determined fractions of SOM (e.g. selective preservation of charcoal), (ii) physical occlusion within stable soil aggregates, (iii) chemical protection by interactions with mineral surfaces or metal ions and (iv) multiple processes shaped by specific environmental conditions.

Selective preservation of recalcitrant compounds is determined by their molecular properties and conformed by molecule size, polarity, ether-bridges, quaternary C-atoms, three-fold substituted N-linkages, phenyl- and heterocyclic N-groups and long chain (hydrophobic) hydrocarbons. However, except for possibly fire neoformed-material termed as black carbon (BC) acquires a poly-condensed aromatic structure, all biomass is decomposed in soil under favourable decomposition conditions.

Stabilization of SOM via their association with silt and clay particles and physical protection afforded by aggregates are mechanisms leading to the accumulation of soil OC in metastable forms. Soil biota may also be involved in the process of SOM occlusion by releasing secretions, root exudates and faunal mucus that act as cementing agents. The aggregates may be (i) constituted by small pore neck sizes and (ii) divided into "compartments" and "walls" which limit movement from different parts of an aggregate and/or (iii) not connected by a continuous water film hindering degradation by enzymes action. Besides this spatial protection can act reducing the diffusion of oxygen into aggregate impeding the activity of aerobic microbes. In fact, it was suggested that in allophanic andosols under perudic conditions, all micropores are filled with water that causes local anoxic circumstances and inhibits SOM decay. The intrinsic hydrophobicity and/or encapsulation

presented by some chemical compounds such as fatty acids reduce the accessibility/activity of microorganisms and/or enzymes.

Interaction with mineral surfaces and metal ions is also assumed to be an important factor in the stabilization of SOM. Hydroxyl groups from carboxylic acids and phenols may be involved in the formation of strong organic-mineral associations. The amphiphilic compounds of SOM are suggested to be strictly associated to mineral surfaces through intimate interactions of proteinaceous compounds that may promote stable associations with minerals. Dissolved organic matter may precipitate by bonds with polyvalent metal cations such as Ca^{2+} and Mg^{2+} in neutral and alkaline soils or be complexed with Al^{3+} and Fe^{2+} and organic anions ($R\text{-}COO^{-}$) in acid soils. Monomeric Al-species (Al^{3+}) are likely associated to microbial toxicity but is considered innocuous once is organically complexed with SOM. Weak interactions (e.g. van der Waals forces) between mineral and organic compounds may act as a relative stabilizer by a temporality fluctuating dipole. The reactivity between OC and minerals is highly dependent of the specific surface. Therefore high specific surfaces of clay-size mineral particles such as silicates, sesquioxides, short-range ordered Fe-oxides and amorphous Al hidroxides are considered highly reactive with organic fraction.

Environmental conditions (temperature, water content, nutrient availability, soil acidity, soil redox state etc.) from distinctive systems/environments will affect the decomposition of SOM. In nature the rates of SOM degradation are fastest under oxidizing conditions, i.e. in the presence of free O_2. Thus water-saturated environment decreases the diffusion of O_2 in soil and impedes the aerobic SOM decay. The anaerobic decomposition (in suboxic/anoxic sistems) of SOM involves slower degradative reactions compared to aerobic systems. This is the case of hydromorphic soils such as mangroves and salt marshes characterized by high OC contents. On the contrary, drought halts the diffusion of extracellular enzymes and dissolved OC in soil, decreasing substrate availability. In regions affected by recurrent fires and long periods of drought, deposition of volatilized hydrophobic molecules can create water-repellency. In highly-porous andosols undergoing through large periods of drought and insolation, hydrophilic groups of SOM suffer a chemical re-orientation that provides them high

hydrophobicity. Hydrophobicity of charred SOM upon fire events has largely been reported. In cold latitudes and/or high altitudes, low temperatures causes the freezing of the soil aqueous phase slowing down the diffusion of substrates and extracellular enzymes within the soil.

Scope and outline of this study

The main objectives of this work were (i) to have a deep understanding of the chemistry of SOM in acid soils that differ in their degree of SOM saturation with Al, (ii) the study of SOM susceptibility to oxidation by chemical reagents of different reactivity ($K_2Cr_2O_7$ and $KMnO_4$) along with the identification of the easily oxidised fractions and/or the resistant fractions and (iii) to obtain an in-depth understanding of specific biogeochemistry processes occurring in highly-reduced hydromorphic soils from a mangrove estuary with special emphasis on their effects on SOM stabilization as well as the impact of the discharge of shrimp farming wastewater on the biogeochemistry of these soils and its implication on OC storing.

In **chapter 2**, the chemical characterization of NaOH-extracted SOM was performed by means of pyrolysis-gas chromatographic-mass spectroscopy (Py-GC/MS). A group of acid soils at early stages of development with variable-charge have been selected: andosols, regosols and umbrisols. The SOM composition of selected alu-andic andosols and cambic umbrisols (alu-humic) has been thoroughly examined by means of Py-GC/MS and compared with that of selected haplic regosols (soils with a podzolizing trend) from the same area. We hypothesized that the SOM stabilization of the former may occur by (i) formation of organo-Al complexes (chemical protection) and/or (ii) SOM encapsulation (physical protection) within microaggregates typical of alu-andic andosols. In **chapter 3**, SOM from a BC-rich colluvial soil (haplic umbrisol) has been oxidized by $K_2Cr_2O_7$ and $KMnO_4$ and the remaining fraction (residue) has been analyzed by Py-GC/MS and solid-state ^{13}C cross polarization-magic angle spinning-nuclear magnetic resonance (solid-state ^{13}C NMR/CPMAS). Thus, the chemical composition of the $K_2Cr_2O_7$– and $KMnO_4$– resistant fraction is elucidated and the susceptibility of SOM from different horizons (from a rich-fresh SOM shallow horizon to deeper horizons with high contributions of BC and microbial SOM) to chemical oxidation is determined. In **chapter 4**, hydromorphic soils (thionic fluvisols) from a semi-arid mangrove-dominated by

Rhizophora and *Avicennia sps.* estuary system in the Northeast Brazilian coast were studied. We compared well-preserved mangroves with OC-poor mangrove sediments affected by rich-nutrient wastewater from shrimp farming in order to determine those biogeochemical processes that may interfere in the OC stabilization in this system.

Summary of the different chapters

Comparison of the mechanisms involved in the stabilization of SOM in soils developed from highly-weatherable materials and in those from low-weatherable rocks under similar temperate climatic conditions

Andosols are largely recognized by their ability to store high contents of OC. They are dark-soils with a characteristic amorphous mineralogy mainly associated to (tephric-) volcanic-derived material but they can also developed from easily-weathering material (e.g. basic rocks as gabbros, basalts and amphiboles). SOM in alu-andic andosols and alu-humic umbrisols is believed to accumulate because of the protection caused through its binding to Al. In **chapter 2**, soils that differed in the abundance of organo-Al complexes were investigated to determine the effect of such interactions on SOM chemistry. For this, the surface horizons of three types of acid soils in the Basque Country (northern Spain) under forest stands were studied: (i) alu-andic andosols (AND soils) on basalts and trachytes, (ii) umbrisols or so-called 'aluminic' (ALU) soils also on basalts and trachytes and (iii) soils with a podzolizing trend (POD), on quartzites. Values of Al extractable with sodium pyrophosphate (Alp) in the surface horizons of these soils ranged between 8.5 and 13.1, 1.9 and 9.3, and 0.8 and 3.7 g kg^{-1} dry weight, for the AND, ALU and POD soils respectively. For POD and ALU soils, surface horizons were sampled at two depths, 0-5 and 5-20 cm, whereas the AND soils were sampled at different depths down to the B horizon. NaOH-extractable SOM from three AND soils, 12 ALU soils and 12 POD soils was studied by pyrolysis-gas chromatography/mass spectrometry. The POD soils had the largest loads of plant-derived markers (lignin, long-chain alkanes and alkenes, methyl ketones, fatty acids); SOM of the AND soils had the smallest amounts of plant-derived SOM and the largest amounts of microbial products (microbial sugars and N-compounds) of the soils studied. ALU soils had an intermediate pattern, as expected. The results indicate that

the SOM of alu-andic andosols, developed from basalt and trachyte rocks, is essentially dissimilar to that of soils derived from quartz-rich parent material, under the same climate conditions and similar forest stands. The dominance of secondary (microbial-derived) SOM in alu-andic andosols, also observed in previous research on sil-andic andosols (these are dominated by short- range ordered Si compounds in contrast to the dominance of organo-Al complexes in alu-andic andosols), reveals the small contribution of primary (plant-derived) material to SOM in soils with andic properties.

SOM resistance towards potassium dichromate ($K_2Cr_2O_7$) and potassium permanganate ($KMnO_4$) oxidation.

The degree of oxidation of the SOM may provide an estimate of its susceptibility to be oxidised by both enzymatic processes of microorganisms and/or electron acceptors present in soil. Between the wide range of chemical oxidants used by the scientific community, potassium dichromate ($K_2Cr_2O_7$) and potassium permanganate ($KMnO_4$) are highly accepted as likely electron acceptors of SOM. The former is considered as a strong acceptor –as has long been used to estimate the OC but also BC content when soils have appreciable contributions of charcoal– and the latter as amenably weaker. $KMnO_4$ has been proposed to be used as an indicator of the labile fraction of SOM based on the assumption that the oxidative capacity of $KMnO_4$ is comparable to that of soil microbial enzymes. Factors such as (i) spatial inaccessibility of organic substrates to the oxidation agent, (ii) complexation reactions with inorganic phases and (iii) the presence of chemically recalcitrant SOM fractions such as charred material can promote low recoveries during oxidation. A difficult standardization of theses methodologies likely arises from the lack of recognition of which SOM fractions susceptible, resistant or partial-resistant to be oxidized. In **chapter 3**, samples from a black C-rich colluvial soil in NW Spain were subjected to $K_2Cr_2O_7$ and $KMnO_4$ oxidation and the residual SOM was NaOH-extracted and analyzed using analytical Py-GC/MS and solid-state ^{13}C CP/MAS NMR in order to study the susceptibility of different SOM fractions (e.g., fresh, microbial, pyrogenic and root-derived) towards these oxidation agents. Non-oxidized samples following the same NaOH-extraction procedure were also analysed. Pyrolysis-GC/MS and ^{13}C

NMR indicated that $KMnO_4$ promotes the oxidation of carbohydrate products, mostly from (i) microbial SOM and (ii) a resistant lignocellulose fraction, causing a relative enrichment of aliphatic moieties and aromatic black C structures. From Py-GC/MS, residual SOM after $K_2Cr_2O_7$ oxidation contained black C, N-containing black C markers and aliphatic structures resistant against this oxidant. This was corroborated by a relatively intense resonance of aromatic C and some signal of alkyl C in ^{13}C NMR spectra. These results confirm that $K_2Cr_2O_7$ oxidation residues contain a non-pyrogenic fraction mainly consisting of long-chain aliphatic structures.

SOM stabilization in hydromorphic soils of semiarid mangrove systems and effect of rich-nutrients wastewaters in the geochemistry.

Regular flooding on mangrove ecosystems reduces the O_2 flux through the sediments and, consequently, anoxic conditions are generated. This displaces aerobic bacteria by anaerobic bacteria. In the absence of O_2, other species (i.e., NO_3^- Fe^{3+}, SO_4^{2-}) are used as electron acceptors by anaerobic microorganisms. However the decomposing activity of the latter decreases as does the free energy resulting from the oxidation of SOM with weaker electron acceptors. These conditions allow mangrove soils (and hydromorphic soils in general) to store high OC contents. However these ecosystems are very sensitive to environmental changes. In **chapter 4**, the main objective of the study was to determine the impact of shrimp farm wastewater effluents (which occupy 26.5% of the study area) on the geochemistry and the OC storage of these soils and estimate the total amount of OC stored in soils of semi-arid mangrove estuary systems. For that, a semi-arid mangrove-dominated –mainly by *Rhizophora and Avicennia sps.*– estuary system in the Northeast Brazilian coast (Ceará State) was selected. The area has a marked seasonality with 8-mo y^{-1} of intense drought. Wastewater affected mangrove was referred to as WAM and undisturbed areas as non-WAM. Redox conditions and OC content were found to be statistically related ($P<0.05$) with seasonality and type of land use (WAM vs. non-WAM). Eh values oscillated from anoxic to oxic conditions (from –5 to 68 mV in WAM and from <40 to >400 mV in non-WAM soils) in the wet season and significantly higher ($P <0.01$) in the dry season (from 66 to 411 mV). OC contents fluctuated between 1.9-

1.1 and 1.4-1.1 kg m^{-2} in the wet and dry season, respectively, for non-WAM, and between 0.8-0.6 and 0.6-0.4 kg m^{-2} in the wet and dry season, respectively, for WAM soils. Iron partitioning was significantly dependent ($P<0.05$) on type of land use, with a smaller degree of pyritization (mean values of 7% vs. 44%) and lower Fe-pyrite presence (mean values of 10 μmol g^{-1} vs. 61 μmol g^{-1}) in WAM soils compared to non-WAM soils. Basal respiration of soil sediments was significantly influenced ($P<0.01$) by type of land use with highest CO_2 evolution rates detected in the WAM soils (mean values of 0.20 mg CO_2 h^{-1}-C g^{-1} vs. 0.04 mg CO_2 h^{-1}-C g^{-1}). We hypothesized that the decrease in OC storage in WAM soils is due to (i) an increase in microbial activity caused by the loading of rich-nutrient effluents and (ii) by an increase of strong electron acceptors [e.g., NO_3^-], that promotes a decrease in pyrite and thus in that of soil OC burial. Stocks of OC in the first 40 cm depth of the semi-arid mangroves from Ceará State (area of 22,936 ha) are estimated to be ~1.5 million t.

SUMARIO

Contexto medioambiental

Las emisiones de gases de efecto invernadero (GHG), tales como el CO_2, CH_4, N_2O y $H_2O_{(g)}$, a la atmósfera han ocurrido de forma natural a lo largo de la historia de la Tierra. Sin embargo, durante los últimos 150 años, actividades antrópicas tales como la combustión de materiales fósiles, uso extensivo de actividades agropecuarias, deforestación y/o el cambio del uso del suelo han aumentado de forma significativa las emisiones de los GHG, especialmente las del CO_2 y el CH_4. Las emisiones de tales gases a la atmósfera son responsables del denominado "forzamiento climático" o también llamado "efecto invernadero". De forma breve, este fenómeno es producido por la absorción de la radiación solar tras reflectar en la superficie terrestre. Como consecuencia, la temperatura media global permanece dentro de un rango que permite la vida en la Tierra. Sin embargo, durante el pasado siglo las emisiones antropogénicas de los GHGs han incrementado sustancialmente, lo que según el IPCC, lleva asociado un incremento de la temperatura.

Estrategias para la mitigación y adaptación al actual forzamiento climático, como por ejemplo el aumento en el secuestro de CO_2 atmosférico a través de la fotosíntesis de la biomasa vegetal, han sido avaladas por agencias tales como el Panel Intergubernamental sobre el Cambio Climático (IPCC). Sin embargo, los suelos pueden acumular más del doble del carbono que el almacenado en la biomasa vegetal y estabilizar el carbono orgánico (OC) con tiempos de residencia mayores que la biomasa vegetal. Por ello, el avance en el estudio y comprensión de los mecanismos implicados en la estabilización de la materia orgánica del suelo (SOM) es necesario para conseguir aumentar los stocks de OC en los suelos y así mitigar las emisiones de CO_2.

Materia orgánica del suelo y su estabilización

El concepto de la estructura de la SOM ha cambiado de forma notoria en los últimos años. La visión histórica la define como un material parcial a altamente policondensado,

amorfo, de color oscuro y de amplio rango molecular. Una percepción más actual sugiere una estructura molecular compuesta por moléculas relativamente simples, cuya composición no necesariamente determina su estabilidad, y que la relativa persistencia de la SOM está principalmente promovida por complejas interacciones entre ésta y el entorno próximo, particularmente con cationes metálicos existentes en la disolución del suelo y componentes coloidales más o menos cristalinos. La SOM es termodinámicamente inestable bajo condiciones óxicas. Su presencia en suelos está explicada por la existencia de condiciones no ideales, de tal forma que el OC es oxidado a CO_2. Estas condiciones no ideales están asociadas a la falta de energía de activación necesaria para tal reacción. Éstas están favorecidas por los diferentes mecanismos de protección de la SOM (p.ej. protección física y química) en el suelo así como con determinadas condiciones ambientales que detienen o reducen la actividad de los microorganismos que actúan como catalizadores de las reacciones de descomposición. Los principales factores implicados en la estabilización de la SOM en suelos son: (i) la recalcitrancia intrínseca de determinadas fracciones de la SOM (p.ej. la preservación selectiva del carbón), (ii) oclusión física dentro de agregados estables del suelo, (iii) protección química por interacciones con superficies minerales o iones metálicos y (iv) múltiples procesos conformados por condiciones ambientales específicas.

La preservación selectiva de los compuestos recalcitrantes está determinada por sus propiedades moleculares y conformada por el tamaño de partícula, polaridad, presencia de puentes éter, C cuaternario, N con tres enlaces substituidos, grupos fenil y grupos N heterocíclicos, e hidrocarburos de cadena larga (hidrofóbicos). Sin embargo, exceptuando aquellos materiales formados durante episodios de incendios y denominados como black carbon (BC) que adquieren una estructura aromática policondensada, toda la biomasa es descompuesta en los suelos.

La estabilización de la SOM a través de sus asociaciones con partículas de limo y arcilla y la protección física proporcionada por los agregados son mecanismos que llevan a la acumulación del OC en el suelo en formas metaestables. La biota del suelo puede también estar envuelta en procesos de oclusión de la SOM mediante la liberación de secreciones, exudados

de raíces y el mucus excretado por la fauna, que actúan como agentes cementantes. Los agregados pueden estar (i) constituidos por poros de muy pequeño tamaño y (ii) divididos en "compartimentos" y "muros" más o menos estancos que limitan los movimientos entre diferentes partes de un mismo agregado y/o (iii) no estar conectados a causa de una capa continua de agua que evita la degradación por la acción de enzimas. Además esta protección espacial puede actuar reduciendo la difusión del oxígeno dentro del agregado impidiendo la actividad de microbios aeróbicos. De hecho, se ha sugerido que en andosoles alofánicos bajo condiciones perúdicas, todos los microporos están rellenos con agua causando condiciones locales de anóxia y por tanto, inhibiendo la degradación de la SOM. La hidrofobicidad intrínseca y/o encapsulación presentada por algunos compuestos químicos, tales como los ácidos grasos, también reduce la accesibilidad/actividad de los microorganismos y/o enzimas.

La interacción con superficies minerales e iones metálicos es también considerado como un factor importante en la estabilización de la SOM. Grupos hidroxílicos de los ácidos carboxílicos y fenoles pueden estar implicados en la formación de fuertes asociaciones órgano-minerales. Los compuestos anfifílicos de la SOM han sido sugeridos por estar estrictamente asociados a superficies minerales a través de íntimas asociaciones con compuestos proteicos que pueden promover asociaciones estables con minerales. La materia orgánica disuelta puede precipitar mediante enlaces con cationes metálicos polivalentes, tales como el Ca^{+2} y el Mg^{+2}, en suelos neutros y alcalinos o ser complejados con el Al^{+3} y el Fe^{+2} y aniones orgánicos ($R\text{-}COO^{-}$) en suelos ácidos. Especies monoméricas del Al (Al^{+3}) están posiblemente relacionadas con toxicidad para los microorganismos pero son consideradas inocuas una vez están complejadas orgánicamente con la SOM. Las interacciones débiles (p.ej. fuerzas de van der Waals) entre los compuestos minerales y los orgánicos pueden actuar como estabilizador mediante la fluctuación temporal del dipolo. La reactividad entre el OC y los minerales es altamente dependiente de la superficie específica del mineral. Por lo tanto las altas superficies específicas de las partículas de tamaño arcilla, tales como los silicatos, sesquióxidos, óxidos de Fe de bajo rango de ordenación e hidróxidos de Al amorfos, son considerados altamente reactivos con la fracción orgánica.

Las condiciones ambientales (temperatura, contenido de agua, disponibilidad de nutrientes, acidez del suelo, estado redox del suelo, etc.) de determinados sistemas/ambientes afectarán a la descomposición de la SOM. En la naturaleza las tasas de degradación de la SOM son más rápidas bajo condiciones oxidantes, i.e. en presencia de O_2 libre. Así en ambientes saturados en agua, disminuye la difusión del O_2 en el suelo e impide la degradación aeróbica de la SOM. La descomposición anaeróbica (en sistemas subóxicos/anóxicos) de la SOM promueve reacciones degradativas más lentas en comparación con sistemas aeróbicos. Este es el caso de suelos hidromorfos, tales como los manglares y las marismas, los cuales están caracterizados por presentar altos contenidos de OC. Por el contrario, condiciones de alta sequedad ralentizan la difusión de enzimas extracelulares y la formación de OC disuelto, disminuyendo así la disponibilidad del sustrato. En regiones afectadas por fuegos recurrentes y largos períodos de sequía, la deposición de moléculas hidrofóbicas volatilizadas pueden crear repelencia al agua. En andosoles con alta porosidad sometidos a largos períodos de sequía e insolación, los grupos hidrofílicos de la SOM sufren una reorientación química que les proporciona una alta hidrofobicidad. La hidrofobicidad de la SOM calcinada a causa de episodios de fuego ha sido muy estudiada. En latitudes frías y/o altas altitudes, las bajas temperaturas causan la congelación de la fase acuosa del suelo ralentizando la difusión de sustratos y enzimas extracelulares dentro del suelo.

Alcance y resumen de este estudio

Los objetivos principales de este trabajo fueron: (i) obtener un incremento del conocimiento de la química de la SOM en suelos ácidos que difieren en el grado de saturación con el Al, (ii) estudiar la susceptibilidad de la SOM a la oxidación mediante agentes químicos de diferente capacidad oxidativa ($K_2Cr_2O_7$ y $KMnO_4$) junto con la identificación de aquellas fracciones fácilmente oxidables y/o aquellas fracciones resistentes, (iii) obtener un incremento del conocimiento de los procesos biogeoquímicos específicos que se producen en suelos hidromórficos altamente reducidos de manglar, con especial énfasis en sus efectos sobre la estabilización de la SOM, así como el impacto de la descarga de aguas residuales generadas

durante la cría de camarones en la biogeoquímica de estos suelos y su implicación en el almacenamiento de OC.

En el **capítulo 2**, la caracterización química de la SOM extraíble con NaOH fue realizada a través de Py-GC/MS (pyrolysis-gas chromatographic-mass spectroscopy; pirólisis-cromatografía de gases-espectroscopía de masas). Un grupo de suelos ácidos incipientes de carga variable han sido seleccionados: andosoles, regosoles y umbrisoles. La composición de la SOM de andosoles alu-ándicos y umbrisoles cámbicos (ali-húmicos) han sido exhaustivamente examinados a través de Py-GC/MS y comparada con la de los regosoles háplicos seleccionados (suelos con tendencia podsolizante) de la misma área. Se parte de la hipóteis de que la estabilización de la SOM de los primeros puede ocurrir por (i) la formación de complejos órgano-Al (protección química) y/o (ii) encapsulación de la SOM (protección física) dentro de los microagregados típicos de andosoles alu-ándicos. En el **capítulo 3**, la SOM de un suelo coluvial rico en BC (umbrisol háplico) ha sido oxidada con $K_2Cr_2O_7$ y $KMnO_4$ y la fracción remanente (residuo) ha sido analizada mediante Py-GC/MS y solid-state ^{13}C NMR/CPMAS (solid-state ^{13}C cross polarization-magic angle spinning-nuclear magnetic resonance; ^{13}C resonancia magnética nuclear en estado sólido de polarización cruzada con rotación de ángulo mágico). De este modo, la composición química de la fracción resistente a $K_2Cr_2O_7$ y $KMnO_4$ es elucidada y la susceptibilidad de la SOM de los diferentes horizontes (desde un horizonte superficial rico en SOM fresca hasta un horizonte más profundo con altas contribuciones de BC y SOM microbiana) a la oxidación química es determinada. En el **capítulo 4**, suelos hidromórficos (fluvisoles tiónicos) de un manglar del semiárido (dominado por *Rhizophora* and *Avicennia sps.*) de la costa nordestina de Brasil fueron estudiados. Se realiza la comparación de manglares bien preservados con sedimentos de manglar pobres en OC y afectados por aguas residuales de la cría de camarón ricas en nutrientes, con el objetivo de determinar aquellos procesos biogeoquímicos que pueden interferir en la estabilización del OC en este sistema.

Sumario de los diferentes capítulos

Comparación de los mecanismos implicados en la estabilización de la SOM en suelos desarrollados a partir de materiales altamente meteorizables y aquellos a partir de rocas poco alterables bajo las mismas condiciones climáticas templadas

Los andosoles son conocidos por su habilidad para almacenar altas cantidades de OC. Son suelos oscuros con una mineralogía amorfa característica asociada principalmente a materiales derivados de la actividad volcánica (téfrico), pero también pueden desarrollarse a partir de material fácilmente metereolizable (p.ej. en rocas básicas como los gabros, los basaltos y las anfibolitas). Se considera que la SOM es acumulada en andosoles alu-ándicos y umbrisoles cámbicos (alu-húmicos) a causa de la protección originada a través de su unión al Al. En el **capítulo 2**, suelos que difieren en la abundancia de complejos órgano-Al fueron investigados para determinar el efecto de tales interacciones sobre la química de la SOM. Para esto, los horizontes superficiales de tres tipos de suelos ácidos del País Vasco (norte de España) bajo bosque fueron estudiados: (i) andosoles alu-ándicos (AND soils) sobre basaltos y traquitas, (ii) umbrisoles o también llamados suelos "alumínicos" (ALU) también sobre basaltos y traquitas y (iii) suelos con tendencia podsolizante (POD), sobre cuarcitas. Los valores de Al extractable con pirofosfato sódico (Alp) en los horizontes superficiales de estos suelos oscilaron entre 8,5 y 13,1, 1,9 y 9,3, y 0,8 y 3,7 g kg^{-1} peso seco, para los suelos AND, ALU y POD, respectivamente. Para los suelos POD y ALU, los horizontes superficiales fueron muestreados a dos profundidades, 0-5 y 5-20 cm , mientras que los suelos AND fueron muestreados a diferentes profundidades hasta el horizonte B. La SOM extraída con NaOH de tres suelos AND, 12 suelos ALU y 12 suelos POD fueron estudiados mediante Py-GC/MS. Los suelos POD tuvieron las mayores cargas de marcadores de plantas (lignina, alcanos y alquenos de cadena larga, metil-cetonas, ácidos grasos); la SOM de los suelos AND presentaron las menores cantidades de SOM derivada de plantas y las mayores cantidades de productos microbianos (azúcares microbianos y compuestos nitrogenados) de los suelos estudiados. Los suelos ALU tuvieron un patrón intermedio como era esperado. Los resultados indican que la SOM de los andosoles alu-ándicos, desarrollados sobre rocas basálticas y traquíticas, es

esencialmente diferente a aquella de suelos derivados sobre materiales originales ricos en cuarzo, bajo las mismas condiciones climáticas y similar vegetación. El predominio de una SOM secundaria (derivada de microbios) en andosoles alu-ándicos, también observada en investigaciones previas sobre andosoles sil-ándicos (estos están dominados por compuestos de Si con bajo rango de ordenación en contraste con el dominio de complejos órgano-Al en los andosoles alu-ándicos), revela la reducida contribución de la SOM primaria (derivada de plantas) en suelos con propiedades ándicas.

Resistencia de la SOM a la oxidación del dicromato potásico ($K_2Cr_2O_7$) y del permanganato potásico ($KMnO_4$).

La determinación del grado de oxidación de la SOM puede ofrecer una estimación de la susceptibilidad de ésta a ser oxidada por la acción de procesos enzimáticos de los microorganismos y/o los aceptores de electrones presentes en el suelo. Entre el amplio rango de oxidantes químicos usados por la comunidad científica, el dicromato potásico ($K_2Cr_2O_7$) y el permanganato potásico ($KMnO_4$) están muy aceptados como aceptores de electrones de la SOM. El primero está considerado como un aceptor fuerte –usado para estimar el OC pero también para la estimación del contenido de BC cuando los suelos tienen apreciables contribuciones de carbón– y el último como sensiblemente más débil. El $KMnO_4$ ha sido propuesto para ser usado como un indicador de la fracción lábil de la SOM, basado en el supuesto de que la capacidad oxidativa del $KMnO_4$ es comparable con aquella producida por los enzimas microbianos del suelo. Factores tales como (i) la inaccesibilidad espacial de los sustratos orgánicos al agente de oxidación, (ii) reacciones de complejación con las fases inorgánicas y (iii) la presencia de fracciones de la SOM químicamente recalcitrantes, tales como el material calcinado, pueden promover bajas recuperaciones durante la oxidación. Una difícil estandarización de estas metodologías probablemente radica en la falta de apreciación/reconocimiento de cuales son las fracciones de la SOM susceptibles, resistentes o parcialmente resistentes a ser oxidadas. En el **capítulo 3**, muestras de un suelo coluvial rico en BC localizado en el NW de España fueron sometidas a oxidación mediante $K_2Cr_2O_7$ y $KMnO_4$ y la SOM residual fue extraída con NaOH y analizada mediante Py-GC/MS y solid-state ^{13}C

CP/MAS NMR a fin de estudiar la susceptibilidad de las diferentes fracciones de la SOM (p.ej., aquella derivada de material fresco, microbiano, pirogénico y aquella derivada de raíces) por la acción de estos agentes de oxidación. Muestras no oxidadas y tratadas siguiendo el mismo procedimiento de extracción con NaOH fueron también analizadas. Pyrolysis-GC/MS y ^{13}C NMR indicaron que el $KMnO_4$ promueve la oxidación de carbohidratos, la mayor parte de (i) SOM microbiana y (ii) una fracción lignocelulósica resistente, causando un relativo enriquecimiento de alifáticos y estructuras aromáticas del BC. A partir de Pyrolysis-GC/MS, la SOM residual después de la oxidación con $K_2Cr_2O_7$ contenía BC, compuestos nitrogenados marcadores de BC y estructuras alifáticas resistentes a este oxidante. Esto fue corroborado por la intensa resonancia del C aromático y la señal del alkyl C en el espectro de ^{13}C NMR. Estos resultados confirman que los residuos de oxidación con $K_2Cr_2O_7$ contienen una fracción no pirogénica que consiste principalmente en estructuras alifáticas de cadena larga.

Estabilización de la SOM en suelos hidromórficos de sistemas de manglares semiáridos y efecto de las aguas residuales ricas en nutrientes en la biogeoquímica del C.

Las inundaciones periódicas producidas en ecosistemas de manglar reducen los flujos de O_2 a través de sedimentos y, consecuentemente, se generan condiciones anóxicas. Esto desplaza las bacterias aeróbicas por bacterias anaeróbicas. En ausencia de O_2, otras especies (p.ej., NO_3^- Fe^{3+}, SO_4^{2-}) son usadas como aceptores de electrones por microorganismos anaeróbicos. Sin embargo la actividad descomponedora de estos últimos decrece como también lo hace la energía resultante de la oxidación de la SOM con aceptores de electrón más débiles. Estas condiciones permiten a los manglares (y suelos hidromórficos en general) acumular altos contenidos de OC. Sin embargo estos ecosistemas son muy sensibles a cambios ambientales. En el **capítulo 4**, el principal objetivo de este estudio fue determinar el impacto de aguas residuales de los efluentes de la cría de camarón (actividad que ocupa el 26,5% del área de estudio) sobre la geoquímica y en el almacenamiento del OC de estos suelos y estimar la cantidad total de OC acumulado en los suelos de sistemas de manglar del semiárido. Para eso, un sistema de manglar del semiárido –dominado principalmente por *Rhizophora* y *Avicennia sps.*– situado en la costa nordestina de Brasil (estado de Ceará) fue seleccionado. El área tiene

una marcada estacionalidad con 8 meses al año de intensa sequía. El manglar afectado por aguas residuales fue referido como WAM (Wastewater-Affected Mangrove; manglar afectado por aguas residuales) y las áreas no alteradas como non-WAM. Las condiciones redox y el contenido de OC están estadísticamente relacionadas ($P<0,05$) con la estacionalidad y el tipo de uso del suelo (WAM frente a non-WAM). Los valores de Eh oscilaron de condiciones anóxicas a óxicas (de –5 a 68 mV en los suelos de WAM y de <40 a >400 mV en suelos de non-WAM) en la estación húmeda y significativamente mayor ($P <0,01$) en la estación seca (de 66 a 411 mV). Los contenidos de OC fluctuaron entre 1,9-1,1 y 1,4-1,1 kg m^{-2} en el período húmedo y seco, respectivamente, para suelos non-WAM, y entre 0,8-0,6 y 0,6-0,4 kg m^{-2} en el período húmedo y seco, respectivamente, para los suelos WAM. La especiación de Fe fue significativamente dependiente ($P<0,05$) del tipo de uso del suelo, con un menor grado de piritización (valores medios de 7% frente al 44%) y menor presencia de Fe-pirítico (valores medios de 10 μmol g^{-1} frente a 61 μmol g^{-1}) en suelos WAM comparado con suelos non-WAM. La respiración basal de los suelos de sedimentos estuvo significativamente influenciada ($P<0,01$) por el tipo de uso del suelo con las mayores tasas de emisión de CO_2 en los suelos WAM (valores medios de 0,20 mg CO_2 h^{-1}-g^{-1} C frente a 0,04 mg CO_2 h^{-1}-g^{-1} C). Se considera como hipótesis que una disminución de la capacidad de acumulación de OC en suelos WAM es fundamentalmente debido a (i) un incremento de la actividad microbiana a causa de una sobrecarga de efluentes ricos en nutrientes y (ii) un incremento de aceptores de electrones [p.ej., NO_3^-], que promueve un descenso en la pirita y de este modo en el OC del suelo enterrado. El stock de OC en los primeros 40 cm de los manglares del estado del Ceará (área de de 22936 ha) fue estimado en ~1,5 millones de toneladas.

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SUMARIO

Contexto medioambiental

As emisións de gases de efecto invernadoiro (GHG), tales como o CO_2, CH_4, N_2O e $H_2O_{(g)}$, á atmósfera aconteceron de forma natural ao largo da historia da Terra. Non obstante, durante os últimos 150 anos, actividades antrópicas tales como a combustión de materiais fósiles, uso extensivo de actividades agropecuarias, deforestación e/ou o cambio do uso do solo aumentaron de forma significativa as emisións dos GHG, especialmente as do CO_2 e o CH_4. As emisións de tales gases á atmósfera son responsables do denominado "forzamento climático" ou tamén chamado "efecto invernadoiro". De forma breve, este fenómeno é producido pola absorción da radiación solar tras reflectar na superficie terrestre. Como consecuencia, a temperatura media global permanece dentro dun rango que permite a vida na Terra. Sen embargo, durante o pasado século as emisións antropoxénicas dos GHGs incrementáronse substancialmente, o que según o IPCC, leva asociado un incremento da temperatura.

Estratexias para a mitigación e adaptación ao actual forzamento climático, como por exemplo o aumento no secuestro de CO_2 atmosférico a través da fotosíntesis da biomasa vexetal, foron avaladas por axencias tales como o Panel Intergubernamental sobre o Cambio Climático (IPCC). Sen embargo, os solos poden acumular máis do dobre do carbono que o almacenado na biomasa vexetal e estabilizar o carbono orgánico (OC) con tempos de residencia maiores que a biomasa vexetal. Por isto, o avance no estudo e comprensión dos mecanismos implicados na estabilización da materia orgánica do solo (SOM) é necesario para conseguir aumentar os stocks de OC nos solos e así mitigar as emisións de CO_2.

Materia orgánica do solo e a súa estabilización

O concepto da estructura da SOM cambiou de forma notoria nos últimos anos. A visión histórica defínea como un material parcial a altamente policondensado, amorfo, de cor escura e de amplio rango molecular. Unha percepción máis actual suxire unha estructura molecular composta por moléculas relativamente simples, cuxa composición non necesariamente

determina a súa estabilidade, e que a relativa persistencia da SOM está principalmente promovida por complexas interaccións entre esta e o entorno próximo, particularmente con catións metálicos existentes na disolución do solo e compoñentes coloidais máis ou menos cristalinos. A SOM é termodinámicamente inestable baixo condicións óxicas. A súa presenza en solos está explicada pola existencia de condicións non ideais, de tal forma que o OC é oxidado a CO_2. Estas condicións non ideais están asociadas á falta de enerxía de activación necesaria para tal reacción. Estas están favorecidas polos diferentes mecanismos de protección da SOM (p.ex. protección física e química) no solo así como con determinadas condicións ambientais que deteñen ou reducen a actividade dos microorganismos que actúan como catalizadores das reaccións de descomposición. Os principais factores implicados na estabilización da SOM en solos son: (i) a recalcitrancia intrínseca de determinadas fraccións da SOM (p.ex. a preservación selectiva do carbón), (ii) oclusión física dentro de agregados estables do solo, (iii) protección química por interaccións con superficies minerais ou ións metálicos e (iv) múltiples procesos conformados por condicións ambientais específicas.

A preservación selectiva dos compostos recalcitrantes está determinada polas súas propiedades moleculares e conformada polo tamaño de partícula, polaridade, presenza de pontes éter, C cuaternario, N con tres enlaces substituidos, grupos fenil e grupos N heterocíclicos, e hidrocarburos de cadea longa (hidrofóbicos). Sen embargo, exceptuando aqueles materiais formados durante episodios de incendios e denominados como black carbon (BC) que adquiren unha estructura aromática policondensada, toda a biomasa é descomposta nos solos.

A estabilización da SOM a través das súas asociacións con partículas de limo e arxila e a protección física proporcionada polos agregados son mecanismos que levan á acumulación do OC no solo en formas metaestables. A biota do solo pode tamén estar envolvida en procesos de oclusión da SOM mediante a liberación de secrecións, exudados de raíces e o mucus excretado pola fauna, que actúan como axentes cementantes. Os agregados poden estar (i) constituidos por poros de moi pequeno tamaño e (ii) divididos en "compartimentos" e "muros" máis ou menos estancos que limitan os movementos entre diferentes partes dun mesmo agregado e/ou

(iii) non estar conectados a causa dunha capa continua de auga que evita a degradación pola acción de encimas. Ademais esta protección espacial pode actuar reducindo a difusión do osíxeno dentro do agregado impedindo a actividade de microbios aeróbicos. De feito, suxeriuse que en andosoles alofánicos baixo condicións perúdicas, todos os microporos están recheos con auga causando condicións locais de anóxia e polo tanto, inhibindo a degradación da SOM. A hidrofobicidade intrínseca e/ou encapsulación presentada por algúns compostos químicos, tales como os ácidos grasos, tamén reduce a accesibilidade/actividade dos microorganismos e/ou encimas.

A interacción con superficies minerais e ións metálicos é tamén considerado como un factor importante na estabilización da SOM. Grupos hidroxílicos dos ácidos carboxílicos e fenoles poden estar implicados na formación de fortes asociacións órgano-minerais. Os compostos anfifílicos da SOM foron suxeridos por estar estrictamente asociados a superficies minerais a través de íntimas asociacións con compostos proteicos que poden promover asociacións estables con minerais. A materia orgánica disolta pode precipitar mediante enlaces con catións metálicos polivalentes, tales como o Ca^{+2} e o Mg^{+2}, en solos neutros e alcalinos ou ser complejados co Al^{+3} e o Fe^{+2} e anións orgánicos ($R\text{-}COO^{-}$) en solos ácidos. Especies monoméricas do Al (Al^{+3}) están posiblemente relacionados coa toxicidade para os microorganismos, pero son consideradas inocuas unha vez están complexadas orgánicamente coa SOM. As interaccións débiles (p.ex. forzas de van der Waals) entre os compostos minerais e os orgánicos poden actuar como estabilizador mediante a fluctuación temporal do dipolo. A reactividade entre o OC e os minerais é altamente dependente da superficie específica do mineral. Polo tanto as altas superficies específicas das partículas de tamaño arxila, tales como os silicatos, sesquióxidos, óxidos de Fe de baixo rango de ordenación e hidróxidos de Al amorfos, son considerados altamente reactivos coa fracción orgánica.

As condicións ambientais (temperatura, contido de auga, disponibilidade de nutrientes, acidez do solo, estado redox do solo, etc.) de determinados sistemas/ambientes afectarán á descomposición da SOM. Na natureza as tasas de degradación da SOM son máis rápidas baixo condicións oxidantes, i.e. en presenza de O_2 libre. Así en ambientes saturados en auga,

disminúe a difusión do O_2 no solo e impide a degradación aeróbica da SOM. A descomposición anaeróbica (en sistemas subóxicos/anóxicos) da SOM promove reaccións degradativas máis lentas en comparación con sistemas aeróbicos. Este é o caso de solos hidromorfos, tales como os manglares e as marismas, os cales están caracterizados por presentar altos contidos de OC. Polo contrario, condicións de alta sequidade retardan a difusión de encimas extracelulares e a formación de OC disolto, disminuíndo así a dispoñibilidade do substrato. En rexións afectadas por lumes recorrentes e longos períodos de seca, a deposición de moléculas hidrofóbicas volatilizadas poden crear repelencia á auga. En andosoles con alta porosidade sometidos a longos períodos de seca e insolación, os grupos hidrofílicos da SOM sofren unha reorientación química que lles proporciona unha alta hidrofobicidade. A hidrofobicidade da SOM calcinada a causa de episodios de lume foi amplamente estudada. En latitudes frías e/ou altas altitudes, as baixas temperaturas causan a conxelación da fase acuosa do solo ralentizando a difusión de substratos e encimas extracelulares dentro do solo.

Alcance e resumo deste estudo

Os obxectivos principais deste traballo foron: (i) obter un incremento do coñecemento da química da SOM en solos ácidos que difiren no grao de saturación co Al, (ii) estudar a susceptibilidade da SOM á oxidación mediante axentes químicos de diferente capacidade oxidativa ($K_2Cr_2O_7$ y $KMnO_4$) xunto coa identificación de aquelas fraccións fácilmente oxidables e/ou aquelas fraccións resistentes, (iii) obter un incremento do coñecemento dos procesos bioxeoquímicos específicos que se producen nos solos hidromórficos altamente reducidos de manglar, con especial énfasis nos seus efectos sobre a estabilización da SOM, así como o impacto da descarga de augas residuais xeradas durante a cría de camaróns na bioxeoquímica destos solos e a súa implicación no almacenamento do OC.

No **capítulo 2**, a caracterización química da SOM extraíble con NaOH foi realizada a través de Py-GC/MS (pirolysis-gas chromatographic-mass spectroscopy; pirólisis-cromatografía de gases-espectroscopía de masas). Un grupo de solos ácidos incipientes de carga variable en estado temperán de desenvolvemento foron seleccionados: andosoles, regosoles e umbrisoles. A composición da SOM de andosoles alu-ándicos e umbrisoles

cámbicos (ali-húmicos) foron exhaustivamente examinados a través de Py-GC/MS e comparada coa dos regosoles háplicos seleccionados (solos con tendencia podsolizante) da mesma área. Pártese da hipótesis de que a estabilización da SOM dos primeiros pode acontecer por (i) a formación de complexos órgano-Al (protección química) e/ou (ii) encapsulación da SOM (protección física) dentro dos microagregados típicos de andosoles alu-ándicos No **capítulo 3**, a SOM de un solo coluvial rico en BC (umbrisol háplico) foi oxidada con $K_2Cr_2O_7$ e $KMnO_4$ e a fracción remanente (residuo) foi analizada mediante Py-GC/MS e solid-state ^{13}C NMR/CPMAS (solid-state ^{13}C cross polarization-magic angle spinning-nuclear magnetic resonance; ^{13}C resonancia magnética nuclear en estado sólido de polarización cruzada con rotación de ángulo máxico). Deste modo, a composición química da fracción resistente a $K_2Cr_2O_7$ e $KMnO_4$ é elucidada e a susceptibilidade da SOM dos diferentes horizontes (dende un horizonte superficial rico en SOM fresca ata un horizonte máis profundo con altas contribucións de BC e SOM microbiana) á oxidación química é determinada. No **capítulo 4**, solos hidromórficos (fluvisoles tiónicos) dun manglar do semiárido (dominado por *Rhizophora* and *Avicennia sps.*) da costa nordestina de Brasil foron estudados. Realízase a comparación de manglares ben preservados con sedimentos de manglar pobres en OC e afectados por augas residuais da cría de camaróns ricas en nutrientes, co obxectivo de determinar aqueles procesos bioxeoquímicos que poden interferir na estabilización do OC neste sistema.

Sumario dos diferentes capítulos

Comparación dos mecanismos implicados na estabilización da SOM en solos desenvolvidos a partir de materiais altamente meteorizables e aqueles a partir de rochas pouco alterables baixo as mesmas condicións climáticas mornas.

Os andosoles son coñecidos pola súa habilidade para almacenar altas cantidades de OC. Son solos escuros cunha mineraloxía amorfa característica asociada principalmente a materiais derivados da actividade volcánica (téfrico), pero tamén poden deselvolverse a partir de material fácilmente metereolizable (p.ex. en rochas básicas como os gabros, os basaltos e as anfibolitas). Considérase que a SOM é acumulada en andosoles alu-ándicos e umbrisoles cámbicos (alu-húmicos) a causa da protección orixinada a través da súa unión ao Al. No **capítulo 2**, solos

que difiren na abundancia de complexos órgano-Al foron investigados para determinar o efecto de tales interaccións sobre a química da SOM. Para isto, os horizontes superficiais de tres tipos de solos ácidos do País Vasco (norte de España) baixo bosque foron estudados: (i) andosoles alu-ándicos (AND soils) sobre basaltos e traquitas, (ii) umbrisoles ou tamén chamados solos "alumínicos" (ALU) tamén sobre basaltos e traquitas e (iii) solos con tendencia podsolizante (POD), sobre cuarcitas. Os valores de Al extractable con pirofosfato sódico (Alp) nos horizontes superficiais destos solos oscilaron entre 8,5 e 13,1, 1,9 e 9,3, e 0,8 e 3,7 g kg^{-1} peso seco, para os solos AND, ALU e POD, respectivamente. Para os solos POD e ALU, os horizontes superficiais foron mostrados a duas profundidades, 0-5 e 5-20 cm , mentras que os solos AND foron mostrados a diferentes profundidades ata o horizonte B. A SOM extraída con NaOH de tres solos AND, 12 solos ALU e 12 solos POD foron estudados mediante pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Os solos POD tiveron as maiores cargas de marcadores de plantas (lignina, alcanos e alquenos de cadena longa, metil-cetonas, ácidos grasos); a SOM dos solos AND presentaron as menores cantidades de SOM derivada de plantas e as maiores cantidades de productos microbianos (azucres microbianos e compostos nitrogenados) dos solos estudados. Os solos ALU tiveron un patrón intermedio como era esperado. Os resultados indican que a SOM dos andosoles alu-ándicos, desenvovidos sobre rochas basálticas e traquíticas, é esencialmente diferente a aquela de solos derivados sobre materiais orixinais ricos en cuarzo, baixo as mesmas condicións climáticas e similar vexetación. O predominio dunha SOM secundaria (derivada de microbios) en andosoles alu-ándicos, tamén observada en investigacións previas sobre andosoles sil-ándicos (estos están dominados por compostos de Si con baixo rango de ordenación en contraste con o dominio de complexos órgano-Al nos andosoles alu-ándicos), revela a reducida contribución da SOM primaria (derivada de plantas) en solos con propiedades ándicas.

Resistencia da SOM á oxidación do dicromato potásico ($K_2Cr_2O_7$) e do permanganato potásico ($KMnO_4$).

A determinación do grao de oxidación da SOM pode ofrecer unha estimación da susceptibilidade desta a ser oxidada pola acción de procesos enzimáticos dos microorganismos

e/ou os aceptores de electróns presentes no solo. Entre o amplo rango de oxidantes químicos usados pola comunidade científica, o dicromato potásico ($K_2Cr_2O_7$) e o permanganato potásico ($KMnO_4$) están moi aceptados como aceptores de electróns da SOM. O primero está considerado como un aceptor forte –usado para estimar o OC pero tamén para a estimación do contido de black C (BC) cando os solos teñen apreciables contribucións de carbón– e o último como sensiblemente máis débil. O $KMnO_4$ foi proposto para ser usado como un indicador da fracción lábil da SOM, basado no suposto de que a capacidade oxidativa do $KMnO_4$ é comparable con aquela producida polos encimas microbianos do solo. Factores tales como (i) a inaccesibilidade espacial dos sustratos orgánicos ao axente de oxidación, (ii) reaccións de complexación coas fases inorgánicas e (iii) a presenza de fraccións da SOM químicamente recalcitrantes, tales como o material calcinado, poden promover baixas recuperacións durante a oxidación. Unha difícil estandarización destas metodoloxías probablemente radica na falta de apreciación/recoñecemento de cales son as fraccións da SOM susceptibles, resistentes ou parcialmente resistentes a ser oxidadas. No **capítulo 3**, mostras dun solo coluvial rico en BC localizado no NW de España foron sometidas a oxidación mediante $K_2Cr_2O_7$ e $KMnO_4$ e a SOM residual foi extraída con NaOH e analizada mediante Py-GC/MS e ^{13}C CP/MAS NMR a fin de estudar a susceptibilidade das diferentes fraccións da SOM (p.ex., aquela derivada de material fresco, microbiano, piroxénico e aquela derivada de raíces) pola acción destos axentes de oxidación. Mostras non oxidadas e tratadas seguindo o mesmo procedemento de extracción con NaOH foron tamén analizadas. Pyrolysis-GC/MS e ^{13}C NMR indicaron que o $KMnO_4$ promove a oxidación de carbohidratos, a maior parte da (i) SOM microbiana e (ii) unha fracción lignocelulósica resistente, causando un relativo enriquecemento de alifáticos e estructuras aromáticas do BC. A partir de Pyrolysis-GC/MS, a SOM residual despois da oxidación con $K_2Cr_2O_7$ contiña BC, compostos nitroxenados marcadores de BC e estructuras alifáticas resistentes a este oxidante. Esto foi corroborado pola intensa resonancia do C aromático e o sinal do alkyl C no espectro de ^{13}C NMR. Estos resultados confirman que os residuos de oxidación con $K_2Cr_2O_7$ conteñen unha fracción non piroxénica que consiste principalmente en estructuras alifáticas de cadea longa.

Estabilización da SOM en solos hidromórficos de sistemas de manglares semiáridos e efecto das augas residuais ricas en nutrientes na biogeoquímica do C.

As inundacións periódicas producidas en ecosistemas de manglar reducen os fluxos de O_2 a través de sedimentos e, consecuentemente, xéranse condicións anóxicas. Esto despraza as bacterias aeróbicas por bacterias anaeróbicas. En ausencia de O_2, outras especies (p.ex., NO_3^- Fe^{3+}, SO_4^{2-}) son usadas como aceptores de electróns por microorganismos anaeróbicos. Sen embargo a actividade descompoñedora destos últimos decrece como tamén o fai a enerxía resultante da oxidación da SOM con aceptores de electrón máis débiles. Estas condicións permiten aos manglares (e solos hidromórficos en xeral) acumular altos contidos de OC. Sen embargo estos ecosistemas son moi sensibles a cambios ambientais. No **capítulo 4**, o principal obxectivo deste estudo foi determinar o impacto de augas residuais dos efluentes da cría de camarón (actividade que ocupa o 26,5% da área de estudo) sobre a xeoquímica e no almacenamento do OC destos solos e estimar a cantidade total de OC acumulado nos solos de sistemas de manglar do semiárido. Para iso, un sistema de manglar do semiárido –dominado principalmente por *Rhizophora* e *Avicennia* sps. – situado na costa nordestina de Brasil (estado de Ceará) foi seleccionado. A área ten unha marcada estacionalidade con 8 meses ao ano de intensa seca. O manglar afectado por aguas residuais foi referido como WAM (Wastewater-Affected Mangrove; manglar afectado por augas residuais) e as áreas non alteradas como non-WAM. As condicións redox e o contido de OC están estadísticamente relacionadas ($P<0,05$) coa estacionalidade e o tipo de uso do solo (WAM frente a non-WAM). Os valores de Eh oscilaron de condicións anóxicas a óxicas (de –5 a 68 mV nos solos WAM e de <40 a >400 mV en solos non-WAM) na estación húmida e significativamente maior ($P <0,01$) na estación seca (de 66 a 411 mV). Os contidos de OC flutuaron entre 1,9-1,1 e 1,4-1,1 kg m^{-2} no período húmido e seco, respectivamente, para solos non-WAM, e entre 0,8-0,6 e 0,6-0,4 kg m^{-2} no período húmido e seco, respectivamente, para os solos WAM. A especiación de Fe foi significativamente dependente ($P<0,05$) do tipo de uso do solo, con un menor grao de piritización (valores medios de 7% frente ao 44%) e menor presenza de Fe-pirítico (valores medios de 10 μmol g^{-1} frente a 61 μmol g^{-1}) en solos WAM comparado con solos non-WAM. A respiración basal dos solos de sedimentos estivo significativamente influenciada ($P<0.01$)

polo tipo de uso do solo coas maiores tasas de emisión de CO_2 nos solos WAM (valores medios de 0,20 mg CO_2 h^{-1}-g^{-1} C frente a 0,04 mg CO_2 h^{-1}-g^{-1} C). Considérase como hipótesis que unha disminución da capacidade de acumulación de OC en solos WAM é fundamentalmente debido a (i) un incremento da actividade microbiana a causa dunha sobrecarga de efluentes ricos en nutrientes e (ii) un incremento de aceptores de electróns [p.ex., NO_3^-], que promove un descenso na pirita e deste modo no OC do solo enterrado. O stock de OC nos primeiros 40 cm dos manglares do estado do Ceará (área de 22936 ha) foi estimado en ~1,5 millóns de toneladas.

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CHAPTER 1

General introduction and approaches to study SOM

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INTRODUCTION

1.1. The global C cycle. The greenhouse effect and climatic forcing

The greenhouse gases (GHGs) –mainly composed by carbon dioxide, methane, nitrous oxide, ozone and water vapour– are the foremost inducers of the climate warming (IPCC, 2001). Between them, CO_2 (with 77% of contribution) and methane (with 14%) are the major GHGs (IPCC, 2007a). Arrhenius (1896) was the first to quantify the contribution of increasing CO_2 concentration to the greenhouse effect and established its involvement in climatic change. In the 20th century, the increasing concentration of GHGs have been unequivocally detected in observational records (Keeling, 1961, 1998; Keeling et al., 1976; MacCracken and Moses, 1982). Keeling et al. (1976) provided a reliable measure of the build-up of atmospheric CO_2 strictly associated to burning of fossil fuel (data recorded on Mauna Loa mount, Hawaii). Later research has focussed in determining (i) the atmospheric abundances of $^{13}CO_2$ isotope, and (ii) the fraction of molecular oxygen (O_2) associated to CO_2 rise to fossil fuel burning (Francey and Farquhar, 1982; Keeling and Shertz, 1992). The CO_2 concentration has increased from 280 ppmv to 380 ppmv since 1850 to 2005 (> 30%) and nowadays it is increasing at a rate of 1.7 ppmv y^{-1} (IPCC, 2007b). Other GHGs, such as methane, have also been measured since 1970 (Steele et al., 1996); measurements have shown a remarkable accelerating rise during the 20th century (Dlugokenchy et al., 1994; Battle et al., 1996). The methane growth rate was estimated from 0.8 to 2% y^{-1} (Dlugokenchy et al., 1994).

The cumulative radiative forcing of anthropogenic GHGs has led to an increase in the average global surface temperature at the current warming rate of 0.15 ºC/decade (IPCC, 2001). As a consequence of this, the sea level has risen (IPCC 2007b) and has led to shifts in ecosystem types (Greene and Pershing, 2007) and promoted changes in the frequency and intensity of wild fires (Running 2006; Westerling et al., 2006). Anthropogenic activities such as land-use change, deforestation, biomass burning, draining of wetlands, soil cultivation and fossil fuel combustion (Lal, 2008) have since long contributed to increments of GHGs emissions (IPCC, 2001). Among several strategies, sequestering CO_2 in soils and biomass is

being considered as a suitable approach to mitigate climate change at least in the short term (Lal, 2008).

1.2. Carbon pools estimation

The global C budget in the Earth is considered constant and C molecules are transferred through different compartments or pools (Houghton, 2007). Lal (2008) designed a widely consulted scheme of main global C pools and interconnections between themselves (Figure 1) having into account: (i) estimations of the major reservoirs of C given by Batjes (1996), Falkowski et al. (2000) and Pacala and Socolow (2004); and (ii) data of fluxes between reservoirs provided by the IPCC (2001). The scheme does not include the inorganic carbon present in the rocks (the inorganic C of the lithospheric pool), which is estimated to be around 60 000 000 Pg of CO_2–C and this represents comprise 99.9% of the total C budget (Falkowski et al. 2000).

The five designated C reservoirs are: oceanic, pedologic, atmospheric and biotic pools and fossil fuel. The pedologic pool is the third largest pool comprising 2500 Pg of CO_2–C (down to 1-meter depth) and it consists of: (i) soil organic carbon (SOC) and (ii) soil inorganic carbon pool (SIC), with estimations of 1550 (Eswaran et al., 1993) and 950 (Schlesinger, 1982) Pg of CO_2–C, respectively. The most accepted estimation of SOC stocks in worldwide soils is that of Eswaran et al. (1993) (~1550 Pg of CO_2–C) although values provided by Batjes (1992) and Sombroek et al. (1993) are also highly accepted. Thus, Batjes (1992) estimated values ranging from 1115 to 2200 Pg, whilst Sombroek et al. (1993) reported total contents of 1220 Pg of CO_2–C. Black-carbon (BC) is not frequently accounted because it is difficult to quantify by conventional methods (Skjemstad et al., 1996; 1999). The accurate global SOC estimation is still difficult to obtain because of: (i) high spatial variability in OC content; (ii) unreliable estimates of area occupied by different types of soils; (iii) unavailability of reliable data (i.e. bulk density: needed to compute volumetric composition); and (iv) the confounding effect of vegetation and land use changes (Eswaran et al., 1993; Batjes, 1996).

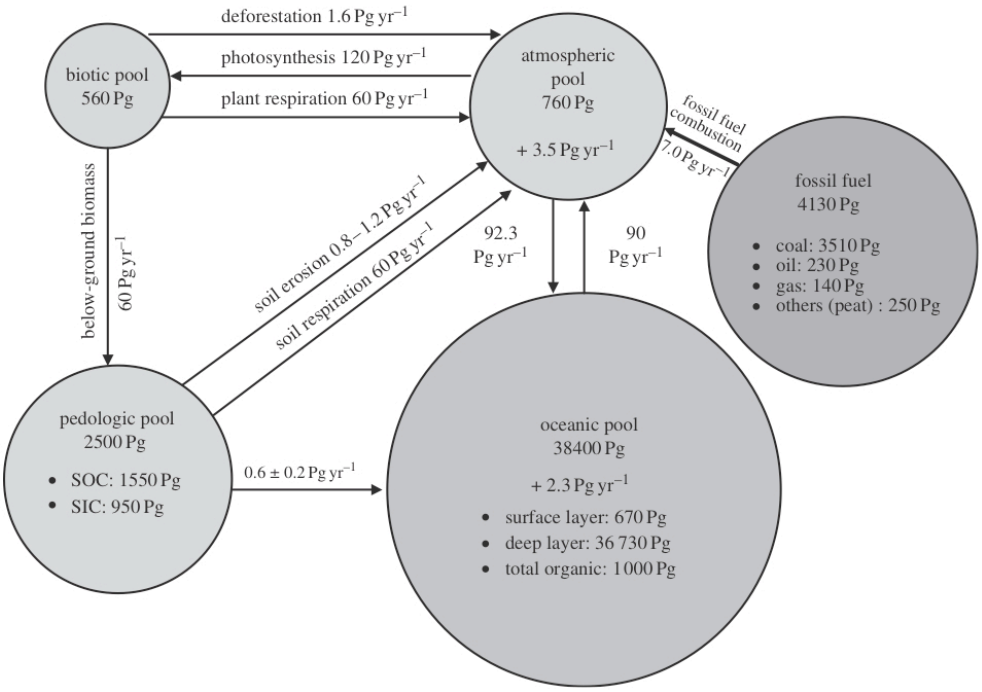

Figure 1. Main global C pools and fluxes between them (Lal, 2008). The data on C pools among major reservoirs are from Batjes (1996), Falkowski et al. (2000) and Pacala & Socolow (2004), and the data on fluxes are from IPCC (2001).

When excluding the lithologic pool, the oceanic pool is considered the largest of the C pools (Lal, 2008) being 15-fold larger than the pedologic pool (38,400 Pg) whilst the pool associated to fossil fuels contains 4,130 Pg CO_2–C (1.6-fold larger than the pedologic pool). The smallest pools correspond to the atmospheric and biotic ones with ~ 1/3 and 1/5 times less CO_2–C stored than the pedologic pool. At present, there is a net flow of 2.3 Pg CO_2–C y^{-1} from the atmosphere into the ocean. In fact the ocean is currently acting as a sink of increasing CO_2 concentrations (natural regulation mechanism) through the displaced CO_2 (g) ←→ CO_2 (aq) towards the aqueous system. In the biotic pool, half of fixed CO_2–C by photosynthesis is depleted by plant respiration and half is accumulated in the pedologic pool as below-ground biomass and later transferred to the atmosphere through soil respiration.

Human activities are increasing the net CO_2 emissions from pedologic, biotic and fossil fuel pools to the atmosphere thus decreasing their C stocks. The 40% CO_2 anthropogenic emissions are mainly caused by burning of coal and fossil oil (Lal, 2008). Besides, agricultural and forestry activities (deforestation, intensive tillage, overgrazing, etc.) involve an important contribution of C transfer from biomass and soils to the atmosphere and have led to the depletion of C stocks in biomass and in soils (Macías and Camps Arbestain, 2010). Thus, deforestation promotes a decrease of 1.6 Pg CO_2–C y^{-1} in the biotic pool and soil erosion triggers releases between 0.8 and 1.2 Pg CO_2–C y^{-1} to atmosphere (Lal, 2008).

1.3. Soil Organic Matter

1.3.1. Definition. Soil organic matter

Roughly, soil organic matter (SOM) encompasses all organic material found in soil – including litter, light fraction (defined in methodology), microbial biomass, water-soluble organic acids and that stabilized by the mineral fraction (Stevenson, 1994)– although some authors exclude non-decayed plant (and their partial decomposition products), animal tissues and living soil biomass (Baldock and Nelson, 2000) and others exclude charcoal (Oades, 1988). However many authors suggested that black C may contribute in the development of SOM (Skjemstad et al., 1996; Golchin et al., 1997; Schmidt et al., 1999). The heterogeneity of SOM –in terms of its source, chemical and physical composition, diversity of function and dynamic– results in a lack of a precise definition and inaccuracy in its structure and reactivity (Piccolo, 1996; Baldock and Nelson, 2000). A new conceptual model determine SOM as a heterogeneous mixture of compounds that display a range of amphiphilic or surfactant-like properties, and are capable of self-organization in aqueous solution (Kleber et al., 2007).

The perception of SOM and its implication on the C cycle has notably changed in the latest years. The historical view was based on the chemical analysis of the extracted materials, generally termed as Humic Substances (HS), which determined the presence of a set of partially of highly polycondensed, amorphous, dark-coloured material with wide range molecular weight (Aiken et al., 1985; Schnitzer, 1978; Piccolo, 1996) that was considered as

the most stable SOM fraction. Actually, HS are determine as a small fraction of SOM and this term is now in disuse (Schmitz et al., 2011). New insights suggest a molecular structure composed by relatively simple molecules, whose composition does not necessarily determines its stability (Kleber et al., 2011), and where the cycling of SOM is governed by its physical and chemical protection and multiple processes shaped by environmental conditions (Figure 2 and 3). Thus, SOM persists not because of the intrinsic properties of the organic matter itself, but because of physicochemical and biological influences from the surrounding environment (Kleber et al., 2011; Schmitz et al., 2011). It has been suggested that multiple variables could conform the structure and composition of SOM. They are summarised as follows: (i) physical protection and interactions with soil minerals playing an important role in its stability over long periods (Eusterhues et al., 2003); (ii) inclusion of roots and mycorrhizal inputs that have high physico-chemical interactions with soil particles (Six et al., 2004); (iii) presence of fired-derived carbon (with a degree of aromaticity affected by the temperature of combustion) that strongly interact with minerals (González-Pérez et al., 2004); (iv) the contribution of deep soil carbon, which includes dissolved organic matter, root products, and transported particulates from the surface to deeper layers –a greater C saturation deficit in deeper layers in comparison with topsoils results in greater sequestration efficiency (Stewart et al., 2008, 2009)– with longer turnover times (Schmitz et al., 2011), (v) microbial activity that may be reduced by suboptimal environmental conditions, nutrient limitation or energy scarcity, and organic matter less accessible because of its sparse density or association with reactive mineral surfaces (Eusterhues et al., 2003; Six et al., 2004; Scheel et al., 2008); (vi) or processes poorly studied such as thawing of permafrost soils (Schmitz et al., 2011). In summary, the C age is not necessarily determined by its molecular structure or thermodynamic stability and it is suggested that the turnover rate is codetermined by the interaction between substrates, microbial organisms and abiotic variables (Kleber et al., 2011).

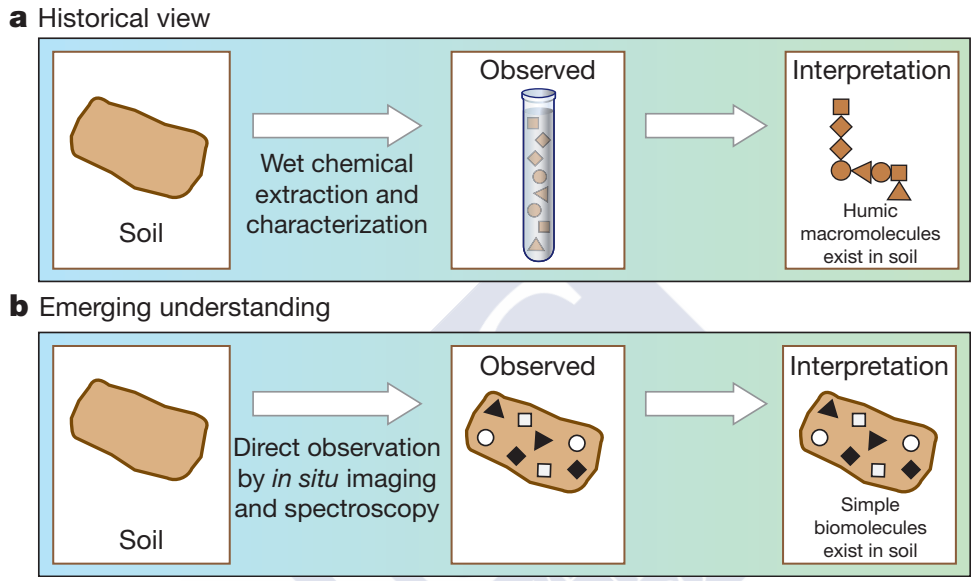

Figure 2. Two different conceptions for the study of the SOM structure (Schmidt et al., 2010). a) The historical view was based on chemical analysis of the extracted materials, generally termed as Humic Substances (HS) whereas b) the emerging understanding of the SOM is determined by direct high-resolution *in situ* observations with non-destructive techniques.

1.3.2. General properties of soil organic matter and associated-effects in the soil

The SOM benefits have long been related to soil fertility (Wander, 2004), although during the past two decades the study of SOM has been gradually focused on its interactions with environmental issues, such as retention of pollutants (Senesi, 1992; Schulten et al., 2001), its role as a source and sink of atmospheric CO_2 and as a GHG emissions mitigation (Batjes et al., 1996; Lal, 2008). The main contribution of SOM on soil properties is summarized according to Stevenson (1994) as follows: SOM (i) acts as source of nitrogen, phosphorous and sulphur for plant and microorganism growth; (ii) constitutes part of the cation exchange capacity (by the presence of –COOH groups) of soils influencing the ability of soils to hold cations (i.e., NH_4^+, Mg^{2+}, Ca^{2+}.); (iii) has an important role in buffering soil pH-fluctuation as well as in reducing elemental toxicity (e.g. Al toxicity) in acid soils (McCallister and Chien,

2000); (iv) contributes to sorption and degradation of organic pesticides; and (v) influences (v) the soil albedo, soil water retention, and soil aeration.

1.4. Soil organic matter stabilization

The SOM accumulated in soil is important in terms of "C sequestration" because it can be long-term stabilized (Sollins et al., 1996; Six et al., 2000). The stabilization mechanisms of SOM in soils lead to large residence times (Torn et al., 1997). In fact, SOM contains more than three times as much carbon as either the atmosphere or terrestrial vegetation (Schmitz et al., 2011). Different types of SOM are linked to different turnover rates: (i) the labile OM pool, that is highly degradable and has a turnover time of 1-2 years in temperate areas and even smaller in tropical soils; (ii) the intermediate OM pool, that comprises the 90% of SOM and degrades within 10-100 years; and (iii) the refractory SOM pool, considered the long-term stabilized OC and has slow decomposition rates and long turnover rates (100 to >1000 years) (Sollins et al., 1996; Six et al., 2000, 2002a; Baldock et al., 2004; Lützow et al., 2006, 2008). Soil C turnover was determined twice as fast in tropical soils compared with temperate soils (Six et al., 2002a). Climate conditions (i.e. temperature and moisture), degree of weathering of primary minerals (i.e. mineralogy) and biota (i.e. specific vegetation: e.g. C_3 versus C_4 plants) of tropical and temperate soils differ widely (Six et al., 2002b; Buurman et al., 2007a). The faster turnover of SOM in the former is extensively ascribed to the higher moisture and temperature. The type of dominant mineralogy in soils from tropical and temperate areas has also an important role on OC-stabilization (Six et al., 2002b). Thus, clay and silt particles with low effective CEC's (cation-exchange capacity) such as 1:1 clay mineral (kaolinites) in tropical regions are less associated with OC and this is therefore less stabilized than 2:1 clays of temperate soils with higher effective CEC.

1.4.1. Stabilization mechanisms of SOM in soils

The proposed stabilization mechanisms of SOM in soils are: (i) selective preservation of recalcitrant compounds, (ii) physical inaccessibility, (iii) interactions with mineral surfaces or metal ions (Davies and Ghabbour, 1998; Six et al. 2002a; Eusterhues et al., 2003; von Lützow

et al., 2006; 2008) and (iv) environmental conditions (e.g. temperature, water availability, soil acidity, soil redox state) (Schmitz et al., 2011) (Figure 3 and 4). The intrinsic recalcitrance of above/below plant-derived material (termed as primary) and microbial-derived (secondary material) define the composition of SOM in topsoil and subsoil, respectively (Buurman et al., 2007a; Schellekens et al. 2009, Buurman and Roscoe, 2010). Molecular properties involved in the primary recalcitrance are: molecule size, polarity, ether-bridges, quaternary C-atoms, three-fold substituted N-linkages, phenyl- and heterocyclic N-groups and long chain (hydrophobic) hydrocarbons. Besides the occurrence of hydrolytic bondings promotes a higher susceptibility to degradation by hydrolases (e.g. cellulose, glucosidase, amidase, pectinase, xylanase, proteases, chitinase) (von Lützow et al., 2006). Besides some authors suggest that soluble mixtures of organic molecules of SOM (with amphiphilic properties) can form organized structures called micelles. These structures consist of hydrophilic exterior regions that shield hydrophobic interiors from contact with water molecules (Kleber et al., 2007). Fire neoformed-material termed as black carbon (BC) acquires a poly-condensed aromatic structure that is long-term stable and highly resistant to decomposition (Pastorova et al., 1994; Sjkemstad et al., 1996, etc.).

Soils consist of particles of sand, silt and clay held together into aggregates of various sizes by organic and inorganic materials. Roots and fungal hyphae fungi stabilize macroaggregates whilst organic residues, bacteria, polysaccharides and inorganic materials (i.e. mineral-oxides) stabilize microaggregates (Tisdall, 1994). Aggregates may occlude SOM particles acting as protection against bacteria and fungi activity. Soil biota may also be involved in the process of SOM occlusion by releasing secretions, root exudates and faunal mucus that act as cementing agents (Oades, 1984, Six et al., 2000). This spatial protection can act at different levels: (i) decrease the access to microorganisms and enzymes and/or (ii) reduce the diffusion of oxygen into aggregate impeding the activity of aerobic microbes (Six et al., 2002a; Lützow et al., 2006). Intercalations of organic molecules into the interlayer spaces of expandable phyllosilicates (2:1) have also been reported (Theng at al., 1986; Kennedy et al. 2002), although Eusterhues et al. (2003) found no evidence of this effect. Hydrophobic properties (although is reversible) of some soils (e.g. andosols with large contents of halloysite

and Fe-rich smectite in Vertisols affected by long periods of drought) (Ugolini and Dahlgren, 2002) and the intrinsic hydrophobicity and/or encapsulation (pseudo-macromolecules) of some chemical compounds (e.g. fatty acids) reduce the accessibility of microorganisms and circumvent the enzymes action (Knicker et al., 2007; Piccolo, 1996).

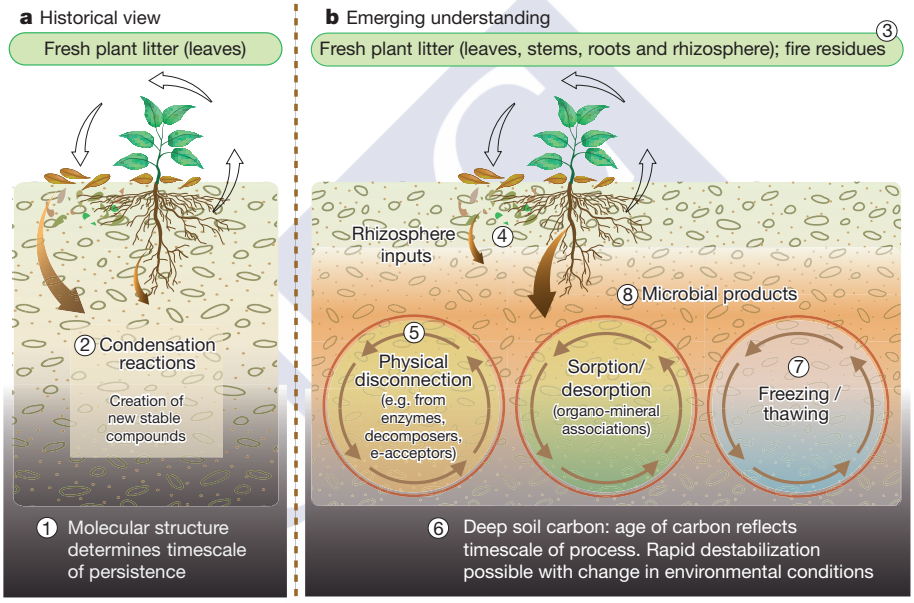

Figure 3. Diagram with the proposed stabilization mechanisms of SOM in soils, contrasting (a) historical and (b) emerging views of soil carbon cycling (Schmidt et al., 2010). In (a), the stable SOM comprise preserved plant inputs and neo-formed products like *humic substances*, whose chemical complexity and composition preserve them from microbial degradation. New insights (b) suggest a molecular structure composed by relatively simple molecules (non-recalcitrant), where the cycling of SOM is governed by its physical and chemical protection and multiple processes shaped by environmental conditions.

SOM sorption to soil minerals and the aforementioned aggregation process are the main factors influencing the stabilization of SOM in subsoil (Eusterhues et al., 2003; Lützow et al., 2006). Groups –OH from carboxylic acids and phenols may form strong organic-mineral associations. The amphiphilicity of SOM fragments is suggested to be involved in organo-mineral interactions as the intimate involvement of proteinaceous compounds that promote stable associations with minerals (Kleber et al., 2007). Dissolved organic matter (DOM) may

precipitate by bonds with polyvalent metal cations (e.g. Ca^{2+} and Mg^{2+}) (Nierop and van Bergen, 2002; Scheel et al. 2008) in neutral and alkaline soils and form strong complexes with Al^{3+} and Fe^{2+} and organic anions ($R\text{-}COO^{-}$) in acid soils (Scheel et al., 2008). The toxicity of aluminium to microorganisms is likely caused by monomeric Al-species (e.g. Al^{3+}), whereas organically complexed-Al is generally assumed to be non-toxic (von Lützow et al., 2006). Nonetheless, Scheel et al. (2008) discarded the effect of Al^{3+} in inhibiting C mineralization given more importance to Al-OM precipitation process. Weak interactions (e.g. Van der Waals forces) between mineral and organic compounds may act as a relative stabilizer by a temporality fluctuating dipole. The large specific surface area increases the reactivity between OC and minerals. Thus, clay-size particles such as silicates, sesquioxides, short-range ordered Fe-oxides and amorphous Al oxides are considered highly reactive (Six et al., 2002a, von Lützow et al., 2006).

Dominance of certain environmental processes can render distinctive systems/environments (e.g., Gleysols), provide a potential capacity to stabilize OC in soils (Davidson and Janssens, 2006): (i) *flooding*, (periodical or permanent) decreases the oxygen diffusion and impedes a direct-oxidation of SOM and aerobic decay activity often allowing only anaerobic decomposition, which involves slower degradative reactions (e.g. wetlands such as mangroves and salt marshes characterized by high OC contents: Chmura et al., 2003; Donato et al., 2011; or peatlands: Gorham, 1991) compared to aerobic systems; (ii) *drought* is caused by a reduction of soil water potential, thus inhibiting diffusion of extracellular enzymes and dissolved OC, and lowering substrate availability at reaction microsites. In fire-prone or drought-prone regions, deposition of volatilized hydrophobic molecules can create water-repellency. For example, in highly-porous andosols, large periods of drought and insolation cause strong hydrophilic groups re-orientation given a high hydrophobic nature with no permanent character (Poulenard et al., 2004). Hydrophobicity of SOM upon fires has largely been reported (Knicker et al., 2007). (iii) *Freezing* causes the diffusion of substrates and extracellular enzymes within the soil to be extremely slow when the extracellular soil water is frozen. For example, in boreal regions it was estimated that large amounts of OC with large residence times (> 30,000 y) are stored under permafrost (Zimov et al., 2006).

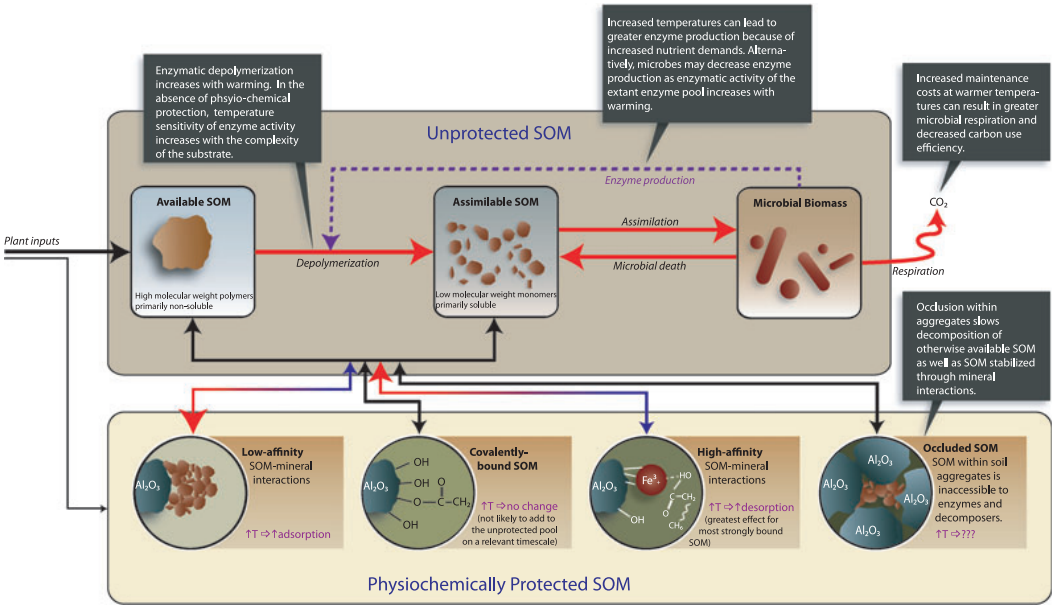

Figure 4. A new conceptual model (Conant et al., 2011) of the SOM decomposition process showing the SOM fluxes between different pools and the stabilization mechanisms implied in the formation of a physiochemical protected SOM as well as the effect of temperature during the course of this process.

1.4.2. Soil organic matter in temperate soils

In worldwide temperate areas, the total SOC (down to 1-m depth) is estimated to be 109 Pg CO_2–C whilst in boreal regions 495 Pg CO_2–C (Batjes, 1999). Temperate and boreal forests store about 161 and 147 Pg CO_2–C, respectively (Schlesinger, 1984). Both temperate and boreal areas, and especially those under deciduous forests, tend to accumulate larger amounts of above ground litter than tropical forests (Vogt et al., 1995). This is mainly attributed to a lower decomposition rate at lower temperatures (Schlesinger, 1984). Furthermore, the occurrence of permafrost in boreal regions promotes a remarkable SOM accumulation with preservation of non-altered/frozen vegetal material during thousand of years (Zimov et al., 2006). Peatlands can also accumulate high amounts of OC at theses latitudes: between 200 and 445 Pg of CO_2–C were estimated in boreal and sub-artic peatlands (Gorham, 1991). They are formed in poorly drained basins and depressions, swamp and marshlands with

shallow groundwater, and highland areas with a high precipitation/evapotranspiration ratio (WRB, 2006)

1.4.2.1. Andic soil properties in soils derived from either volcanic ash or easily-weatherable minerals: andosols

Dark SOM-rich andosols are mainly associated to (tephric-) volcanic material (Parfitt and Kimble, 1989; Shoji et al., 1993) at early stages of weathering but they can also develop on easily-weathering material (e.g. basic rocks as gabbros, amphiboles, granulites: Macías et al., 1978; García-Rodeja et al., 1987; Verde et al., 2005; amphibole schists: Camps Arbestain et al., 2001 or feldspar-rich granite: Delvaux et al., 2004) under highly leaching conditions. They occupy a small part of the Earth's land surface (1-2%) and are mainly formed (>90%) under humid climate (high weathering-induction) (Wilding, 2000). The vast part of reports focused on the humid-temperate environment while those from tropical, arid and cold regions have been less studied (Dahlgren et al. 2004). Andosols are considered the mineral soil order that have the largest accumulations of SOM among (Ugolini and Dahlgren, 2002; Dahlgren et al. 2004). The OC storage/stabilization, low bulk density, variable charge, high water-holding capacity, thixotropy and phosphate retention are some characteristics often reported for these soils (Shoji et al., 1993; Ugolini and Dahlgren, 2002). The fast weathering of the parent material of these soils (tephra or volcanic glass, basalt, amphibolite, etc.) promotes a fast release of dissolved Al, Si and Fe, their over-saturation in solution and the subsequent rapid precipitation in short-range order minerals (e.g. Al/Si-derived: allophane, imogolite and halloysite or Fe-derived: ferrihydrite) and/or organo-metallic complexes (Shoji et al., 1993; Macías et al., 2008). Basically, poorly crystalline minerals are formed as the rate of weathering is faster than the rate of crystallinization (Macías et al., 1978). These poorly crystalline clay minerals –described as aggregated spherical or tubular particles with large surface area– have a high surface charge (pH-dependent) and a large anion retention capacity (Wada, 1989; Dahlgren et al., 1993). Andosols are divided depending on whether they are allophanic (silandic), metal-humus complex dominated (aluandic) or vitric (Shoji, 1985; Ugolini and Dahlgren, 2002). The rate of cation release, pH of the soil solution and SOM content will

largely determine whether allophanic andosols or soils dominated by organo-metallic complexes are formed (Ugolini and Dahlgren, 2002). Allophane formation is favoured in soils with a pH>4.9 whilst in soils with pH values < 4.9 promote the formation of metal-OM complexes is dominant. Acid media also promote the 2:1 phyllosilicates formation (Shoji, 1985). In sil-andic andosols, allophane and imogolite form stable organic-mineral complexes through: (i) anion and inner-sphere ligand-exchange reactions and (ii) physical protection by aggregated spherical particles (Buurman et al., 2007a). In alu-andic andosols, Al/Fe-OM complexes have been shown to be very stable against biodegradation (Nanzyo et al., 1993), and more stable than allophane-OM complexes (Boudot, 1992). Metal-OM complexes can be resistant for hundreds to thousand of years (Six et al., 2002a). The reduced bacterial activity that results from the presence of free Fe, Al and/or allophane, low soils pH (in alu-andic andosols) and poor availability of phosphorus may be also entailed in the SOM preservation in these soils (Buurman et al., 2007a; Naafs, 2004). Furthermore, micro-aggregates –with small pores and a large water-holding capacity– formed in allophanic andosols under perudic conditions may generate local anoxic circumstances that inhibits SOM decay (Buurman et al., 2007a). In the **chapter 2**, we performed a detailed study of the SOM chemistry of (i) alu-andic soils (developed in temperate latitudes) from acid eutrophic volcanic material (images of the studied soils are illustrated in Figures 5 a-c and 6 a-c).

1.4.2.2. Quartzitic material-derived temperate soils (dystrophic): podzols

Theses soils are separated in boreal (or also the so-called zonal) and non-boreal (or intrazonal) podzols (photograhs of boreal and non-boeal podzols are shown in Figure 7 a,b). The formers occur in cold climates at high latitudes and altitudes, and the latters, both well-drained and poorly drained podzols, are evolved on poor parent material in non-boreal latitudes (even in tropics) (Buurman and Jongmans, 2005). They have a distinctive vertical pedological-sequence (simplified): O-(Ah)-E-Bhs-C i.e. (i) a dark-partly humified-O horizon (histic) and (ii) an Ah horizon that may be absent in most boreal podzols; (iii) an eluviated-E (albic) horizon, (iv) an spodic Bhs horizon and (v) a C saprolitic-horizon (WRB, 2006). The most

Figure 5 a-d. *Aluminic soils* (0–5 and 5–20 cm) –classified as cambic umbrisols (alu-humic), haplic or leptic, depending on thickness, and umbric leptosol (humic, dystric) (WRB, 2006)– were submitted to study (ALU soils) in the chapter 2. They were collected in Moint Karakate (Basque Country, Spain), a volcanic massif with dominant basalt and trachyte. These soils were under pine (*Pinus radiata* D. Don), oak (*Quercus robur* L.), beech (*Fagus sylvatica* L.), *Podocarpus spp*. and *Chamaecyparis spp*. stands. Photographs 2 a and 2 b were taken of soils under basalts and 2c and 2d under trachytes (pictures provided by NEIKER-Instituto Vasco de Investigacion y Desarrollo Agrario, Departamento de Agroecosistemas y Recursos Naturales).

Figure 6 a-d. A group of alu-andic andosols (dystric, thixotropic) (WRB, 2006), also referred as AND soils, were included in the study displayed in the chapter 2. They were collected in Moint Karakate (Basque Country, Spain), a volcanic massif with dominant basalt and trachyte. The AND soils were sampled down to the B horizon, and subdivided according to their morphological features into two subhorizons, whose depths varied depending on the soil profile reaching a depth of 100/135 cm. These soils were under pine (*Pinus radiata* D. Don), oak (*Quercus robur* L.), beech (*Fagus sylvatica* L.), *Podocarpus spp*. and *Chamaecyparis spp*. stands. The photograph 3 a was taken of a soil under trachytes, 3 d was taken of a soil under basalts and 2 b and 2 c under both trachytes and basalts (pictures provided by NEIKER).

remarkable processes of the SOM transportation and subsequent accumulation in the spodic-B horizon (Bh/Bhs) of podzols are (i) the formation and downward transport/illuviation of sesquioxides-organic acids complexes, (ii) the contribution of root SOM, especially in those evolved on nutrient-rich parent materials that have fast organic matter dynamics and (iii) a larger abundance of DOC-derived organic matter layers in those on nutrient-poor parent materials and under hydromorphic that promotes a slow organic matter turnover (Lundström, 1993; Buurman and Jongmans, 2005). In surface, leaching from plants, decomposition of litter and exudation by roots and microorganisms produces high amounts of organic acids (Lundström, 2000), that promotes redissolution of SOM-metal complexes at the top of the B horizon, and reprecipitation at greater depths (Dahlgren and Ugolini, 1989; Lundström, 1993). This organic-sesquioxides illuviation forms a coarse-textured weathered cation-impoverished eluvial-E (albic) horizon. Cation-rich soils with high contents of exchangeable cations induce the metal flocculation avoiding the E-horizon formation (Lundström, 2000). The SOM-rich spodic-B horizon (Bh/Bhs) contains a further increase of Al and Fe by precipitation of the OM-sesquioxides complexes from upper layers or by additional contributions from roots (Buurman et al., 2005). The likely causes of the OM- sesquioxides precipitation can be by: (i) microbial decomposition of the SOM in the Bhs horizon that would decrease the C/metal ratio and induce their precipitation (Schnitzer, 1969) or (ii) interaction with Al-rich residues in Bs horizon (Dahlgren and Ugolini, 1989). In well-drained boreal podzols the accumulation of sesquioxides from overlying layers can be considerable, while SOM accumulation is much less because their high turnover. On the contrary, in nutrient-poor environments without mesofauna activity, i.e. hydromorphic podzols, the root-derived SOM shifts to illuvial SOM causing strong humus accumulation (Buurman and Jongmans, 2005).

The chemistry of SOM in the surface horizons of podzols is strongly influenced by the slow decay of fresh organic detritus. Studies using pyrolysis-GC/MS show the dominance of lignin moieties and polysaccharides –with an important presence of levoglucosan from relatively intact polysaccharides and a low contribution of microbial carbohydrate products– in the surface horizons (Buurman et al., 2005). Plant-derived aliphatic polymers and easily degraded microbial polysaccharides, as well as highly degraded lignin moieties, are found in

Figure 7 a,b. Photographs of (a) a boreal podzols from The Nederlands and (b) a non-boreal podzol from Brazil. The photograph 9 a contains a well-drained podzol from Galgenberg, The Nederlands. This profile has a mixed vegetation of *Pinus, Betula, Sorbus* and *Rubus sp.* (photograph by courtesy of Judith Schellekens). The photograph 9 b correspond with a tropical 2 m thick podzol from Brazil (provided by Pablo Vidal-Torrado).

Figure 8 a-d. Surface samples (0–5 and 5–20 cm), referred as POD soils or soils with a *podzolizing* trend in the chapter 2, were collected in a quartzitic massif: Moint Oiz (Basque Country, Spain) and were classified as haplic regosols (dystric) (WRB, 2006) These soils were sampled under *Pinus radiata* D. Don (pictures provided by NEIKER).

the eluvial E-horizons (Buurman et al., 2005; 2007b). The relative abundance of aliphatic polymers decreases in the upper B-horizons, but increases in deeper B-horizons where roots decay (Buurman et al., 2005). A higher microbial activity has been detected in B-horizons, indicated by a high content of hopanoids, branched C_{15} and C_{17} fatty acids, and microbial polysaccharides, as well as small phenols and aromatic compounds (Buurman et al., 2005). In well-drained podzols, organic inputs in the B-horizons are mainly from roots, whilst in waterlogged horizons they originate from dissolved organic matter (Buurman et al., 2005). In **chapter 2** SOM of incipient podzols were characterized using Py-GC/MS technique to further deepen the knowledge in this area (see photograhs of studied podzols in Figure 8 a-d).

1.4.3. Soil organic matter in tropical soils

According to Eswaran et al. (1993) tropical soils store 506 Pg CO_2–C (32% of the global soil OC) 206 Pg CO_2–C of these being assigned to forest soils. In tropical latitudes (between 25°N and 30°S), a system of salt-tolerant forest species termed *mangroves* is located in areas of the coastline as fringe-bays, estuaries and lagoons, and in inland areas following the basin-rivers (Valiela et al., 2001). This system is well-known by its large efficiency in the OC storage (Neue et al., 1997; Bouillon et al., 2008; Kristensen et al., 2008; Donato et al., 2011). It is estimated that worldwide mangroves accumulate between 4 to 20 Pg CO_2–C containing on average of 1,023 Mg CO_2–C ha^{-1} (Donato et al., 2011). Several researchers refer to mangroves by their capacity to store OC –together with saltmarshes and seagrass–as sinks of *blue carbon* (Chmura et al., 2003; Duarte and Cebrián, 1996; Duarte et al., 2005; Bouillon et al., 2008). Mangroves are distributed in 118 countries (Giri et al., 2011) with an extension of 137,760 km^2. Indonesia has the largest area with the 22.6% of global total followed by Australia, Brazil, and Mexico (7.1%, 7.0% and 5.4%, respectively) (Giri et al., 2011).

1.4.3.1. Flooding-affected soil sediments: mangroves

Riverine mangrove systems are influenced by regular oscillations of tides that reduce the O_2 flux –reported as a strong oxidant of SOM in oxic marine substrates (Equation 1) (Huerta-Diaz and Reimer, 2010)– through the sediments prevailing anoxic conditions, mainly in depth

(Otero and Macias, 2002; Otero et al., 2006; Ferreira et al., 2007). Thus, organic matter is highly stabilised in these anoxic sediments by impairment of aerobic-microbial activity although anaerobic bacteria keeps decomposing, but at much slower rate (Alongi et al., 2004; 2005). In the absence of O_2 and excluding fermentation, other species (i.e., NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-} and CO_2) are used as electron acceptors (Froelich et al., 1979) by anaerobic microorganisms following a sequential pattern and being SO_4^{2-} and CO_2 the last ones to act (Berner, 1984; Otero and Macías, 2002). In anoxic environments the reduction of Fe(III) and sulphate are the main processes involved in the oxidation of OC to CO_2 (Huerta-Diaz and Reimer, 2010) (Equation 2 and 3).

$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)+138O_2 \Rightarrow 106CO_2+16HNO_3+H_3PO_4+122H_2O$ (1)

$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)+424Fe(OH)_3+756CO_2 \Rightarrow 424Fe_2^{+}+862HCO_3^-+16NH_4+HPO_4^{2-}$ $+304H_2O$ (2)

$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)+53SO_4^{2-} \Rightarrow 106HCO_3^-+16NH_3+53H_2S+H_3PO_4$ (3)

$Fe^{+2}+HS^- \Rightarrow FeS+H^+$ (a) followed by $FeS+H_2S \Rightarrow FeS_2+H_2$ (b) (4)

$FeS_2+7/2O_2+H_2O \Rightarrow Fe^{2+}+2SO_4^{2-}+2H^+$ (5)

$FeS_2+14Fe^{3+}+8H_2O \Rightarrow Fe^{2+}+2SO_4^{2-}+16H^+$ (6)

$5FeS_{2(s)} + 14NO_3^-{}_{(aq)} + 4H^+{}_{(aq)} \Rightarrow 7N_{2(g)} + 10SO_4^{2-}{}_{(aq)} + 5Fe^{2+}{}_{(aq)} + 2H_2O_{(aq)}$ (7)

Active processes of pyrite formation/oxidation –in recent sediments or by changes in the geochemical conditions– promote the OC oxidation in marine sediments (Berner and Raiswell, 1983; Huerta-Diaz and Reimer, 2010). Indeed pyrite is likely associated to the burial of SOM (Berner, 1982; Berner and Raiswell, 1983). Briefly, pyrite is formed by interaction of Fe(II) and hydrogen sulphide (H_2S) (Equation 4a and b). The former is produced through Fe(III) (crystalline Fe-oxyhydroxides) bacterial reduction (Equation 2) and the latter by either SO_4^{2-} reduction (Equation 3) or through SOM decomposition (both mediated by anaerobic bacteria) (Berner, 1970, 1984, Canfield, 1989). Both processes promote a strong OC oxidation (Berner, 1982, Huerta-Diaz and Reimer, 2010). A metastable fraction known as acid volatile sulphides (AVS) is formed during pyrite formation (Equation 4a). This fraction is later transformed into pyrite (FeS_2) (Equation 4b) (Candfield, 1989). Pyrite is oxidised when it undergoes oxic

conditions (Equation 5). Large amounts of Fe(III) also promotes pyrite oxidation (Equation 6) (Moses et al., 1987). Thus, oxidation of pyrite by either O_2, Fe^{3+} (Huerta and Reimer, 2010) and/or nutrients in solution such as NO_3^- (Schippers and Jørgensen, 2002; Jørgensen at al., 2009) increases the SO_4^{2-} levels in sediments promoting the bacterial SO_4^{2-} reduction (with SO_4^{2-} acting as electron acceptor) with the concomitant OC oxidation (Equations 5, 6 and 7). This process is likely shown by an increase of DOP (degree of pyritization; ratio calculated as Fe-pyritic/Fe-reactive + Fe-pyritic and given in %; Berner, 1970) and an increase of AVS because the formation of pyrite is still active. In the **chapter 3**, we studied the likely oxidation of pyrite by inputs of (i) nitrate-rich wastewaters and (ii) increment of microbial activity by effluents of shrimp farms in mangrove areas and determine whether the OC storage capacity of studied sediments is consequently affected (illustrations of the studied mangrove soils are represented in Figure 9 a-d).

2. APPROACHES TO STUDY SOM

2.1. SOM fractionation prior analysis

A broad set of animal- and vegetal-derived materials conform a complex SOM that is subject to different degrees of decomposition and is linked to the mineral matrix in different states of association (Buurman and Roscoe, 2010). Thus, SOM can be (i) weakly associated to mineral particles (referred to as light fraction) and found either as free-associated/uncomplexed (FLF, free light fraction) or encapsulated (OLF, occluded light fraction) during aggregation process; and (ii) highly associated to minerals forming organo-mineral complexes (OMC) (Christensen, 2001; Buurman and Roscoe, 2010). The most common spectroscopic techniques used for the characterization of soil OC (e.g., solid-state ^{13}C NMR, analytical pyrolysis) require a fractionation/extraction and purification of SOM prior to these analyses in order to avoid interferences (Schnitzer and Schulten, 1992). Indeed, minerals (e.g. Al- and Fe-oxides) promote distortions in the paramagnetic signal in solid-state ^{13}C NMR (Knicker, 2011) or abrupt interferences in pyrolysis spectra as well as they may be involved in catalytic reactions during pyrolysis with formation of artefacts (Schnitzer et al., 1994). According to Stevenson (1994) and Schnitzer and Schuppli (1989) the ideal extraction method should meet

Figure 9 a-d. Illustrations of the studied mangroves and surrounding areas located in a semiarid region in the state of Ceará (north-eastern Brazil). Photographs 10 a,c correspond with well-preserved mangroves dominated by *Rhizophora mangle* L. The photograph 10 c shows the sampling procedure of mangrove sediments in areas without human disturbance. The picture 10 b corresponds with areas of well-preserved mangroves under *Avicennia schaueriana* L. The photograph 10 d illustrates the typical vegetation and common soils (planosols, WRB) of this semiarid region that restricts mangrove forests to coastal areas. Here, the vegetation is dominated by carnauba palms (*Copernicia prunifera*) and soils can accumulate water in surface for a long time by presence of a slowly permeable subsoil (pictures taken by the author).

the following requirements: (i) lead to the isolation of unaltered compounds; (ii) the extracts should be free of inorganic contaminants (e.g. clay and polyvalent cations); (iii) all fractions must be represented; (iv) the method should be universally applicable to all soils; (v) the extraction method must be relatively simple and (vi) the method should not be too time-consuming so that large numbers of soil samples can be handled.

The classical chemical fractionation of SOM is based on aqueous solubility in alkali reagent (e.g., NaOH) and therefore does not reflect a clear separation based in a well-defined structure or composition (Sáiz-Jiménez and de Leeuw, 1986; Sutton and Sposito, 2005). NaOH extraction is nowadays used as extractant of a representative fraction of SOM (alkali-soluble extractable fraction) (Schnitzer and Schuppli, 1989; Buurman and Roscoe, 2010). However, it has been criticized as it may modify SOM composition through hydrolysis and autooxidation (Stevenson, 1994), although there is little evidence on the damage or modification of SOM by dilute alkali (Schnitzer and Schuppli, 1989). Neutral salts, such as $Na_4P_2O_7$ are also used to extract soil SOM by forming insoluble precipitates or soluble complexes with Ca, Fe, Al and other polyvalent cations (Bremner and Lees, 1949). Comparatively, 0.1 M NaOH solution enables the extraction of more high-molecular weight and less-aromatic SOM than does 0.1 M $Na_4P_2O_7$ solution (pH 7) (Schnitzer and Schuppli, 1989). Therefore, SOM soluble in NaOH comprises highly altered plant and microbial residues whilst the non NaOH-soluble fraction would include less decomposed plant fragments with larger size, microbial cellular lipids, charcoal and that SOM stabilized by clay bonds and/or encapsulated into aggregates. Not withstanding $Na_4P_2O_7$ could extract both free SOM and organomineral complexes without Al-, Fe- removal (Schnitzer and Schuppli, 1989). Other reagents such as $H_2C_2O_4$ (acid oxalate), commonly used to dissolve short-range order Al hydroxides and oxihydroxides, Al-bonded to organic matter and in allophane and imogolite (García-Rodeja et al., 2004) would extract an important fraction of SOM in e.g. andosols. This reactant has not yet been characterized. Multitudes of methods were proposed to SOM fractionation throughout of sequential extractions of acid/base precipitation/solubilisation together with organic solvents and/or particle size fractionation (i.e. Hayes, 1986; Schnitzer and Schuppli, 1989, von Lützow et al.

2007). A consensus in what methodologies are needed to apply previous to spectroscopic assays will optimize the SOM characterization studies in order to obtain comparable studies.

2.2. Analytical approaches for characterizing soil organic matter

Structural information on soil organic matter (SOM) at the molecular level can be obtained by means a set of analytical techniques that can be cited as follows: (i) chemolytic assays plus colorimetric and/or GC MS analyses that are focused in determination of specific groups of compounds (i.e. polysaccharides, proteins, lipids, etc.) (Kögel-Knabner, 1995) or procedures based on the determination of SOM lability/recalcitrance towards oxidants (Loginow et al., 1987; Blair et al., 1995; Heanes, 1984); (ii) physical fractionation into organo-mineral fractions based on particle size and/or density yields (Christensen, 1992) or those able to study macromolecular structures such as (ii) thermochemolytic or the so-called pyrolysis analyses coupled in the most of the cases to GC/MS (Sáiz-Jiménez and de Leeuw, 1986, Ralph and Hatfield, 1991) and (iv) non-destructive spectroscopic techniques such as (1) solid-state ^{13}C nuclear magnetic resonance spectroscopy (Kögel-Knabner, 1997; 2000), (2) infra-red (IR) and (3) electron spin resonance (ESR). Py-GC/MS and solid-state ^{13}C NMR are currently the most used in the determination of SOM chemical composition.

The chemolytic procedures commonly used for SOM characterization (Kögel-Knabner, 1995) are cited as follows: (i) determination of total polysaccharides by a two-step selective hydrolytic depolymerisation reaction followed by colorimetric reaction (Pakulski and Benner, 1992); (ii) characterization of the amount and degree of lignin degradation by CuO oxidation (Hedges and Ertel, 1982) and later identification/quantification by HPLC or gas-chromatography; (iii) total amino sugars determination (microbial-derived) by acid hydrolysis and colorimetric determination (Scheidt and Zech, 1990); (iv) extractable lipids by mixture of chloroform and methanol (Bligh and Dyer, 1959) and (v) hydrolyzable proteins (amino acids) by acid-hydrolysis and colorimetric determination (Stevenson and Cheng, 1970). Although some of the aforementioned procedures are currently in disuse (Kögel-Knabner, 2000) others are currently highly used: e.g. Naafs el al. (2004), and Jansen et al. (2006) reporting results from solvent-extractable lipids fraction. The SOM characterization according to the lability

index is performed by a set of chemical oxidants such as: potassium dichromate ($K_2Cr_2O_7$) (Heanes, 1984; Tabatabai, 1996; Skjemstad and Taylor, 1999), potassium permanganate ($KMnO_4$) (Loginow et al., 1987; Blair et al., 1995; Tirol-Padre and Ladha, 2004), H_2O_2 (Eusterhues et al., 2005), $Na_2S_2O_8$ (Eusterhues et al., 2003) or NaOCl (Kleber et al., 2005). In the **chapter 2**, $K_2Cr_2O_7$ and $KMnO_4$ residues are analysed by Pyrolysis-GC/MS and soli-state ^{13}C NMR with the objective to determine the susceptibility of different SOM fractions towards the aforementioned oxidation agents (photograph and profile representation of the studied BC-rich soil in Figure 10).

2.2.1. Solid-state ^{13}C NMR spectroscopy

Solid-state ^{13}C NMR is a non-invasive method that offers us an overview, although with a limited resolution, on the SOM structures present in soil (Kögel-Knabner, 1997). It provides information on the distribution of C in functional group classes or chemical shifts regions based on differences in resonance energy of ^{13}C nuclei in an artificial electromagnetic field, which depend on the arrangement of the surrounding atoms and groups (Kögel-Knabner, 2000; Baldock and Smernik, 2002, Knicker, 2011). To enhance signal intensity and reduce analysis time, solid- state ^{13}C NMR is often employed in CPMAS (cross-polarisation magic angle spinning) mode. This technique transfers resonance energy from the abundant ^{1}H to the dilute ^{13}C spins (Kögel-Knabner, 1997). Although often discussed to underestimate Black carbon, recent studies demonstrated that most charcoals have an atomic H/C ratio > 0.5 and thus provide sufficient protonation for efficient cross polarization and reliable NMR spectra (Knicker et al., 2005a). In addition, this technique allows the semi-quantification of the aforementioned functional groups by the integration of the signal intensity in their respective chemical shift regions. Spectra may be divided into different regions of chemical shift. Thus, according to Knicker et al. (2005b): (i) the region between 0-45 ppm is assigned to alkyl-C corresponding to terminal methyl groups and methylene groups of aliphatic moieties; (ii) the O-alkyl C region is typically assigned to carbohydrate-derived structures, placed between 45-95 ppm; (iii) between 90 and 160 ppm resonance lines of olefins and aromatic C

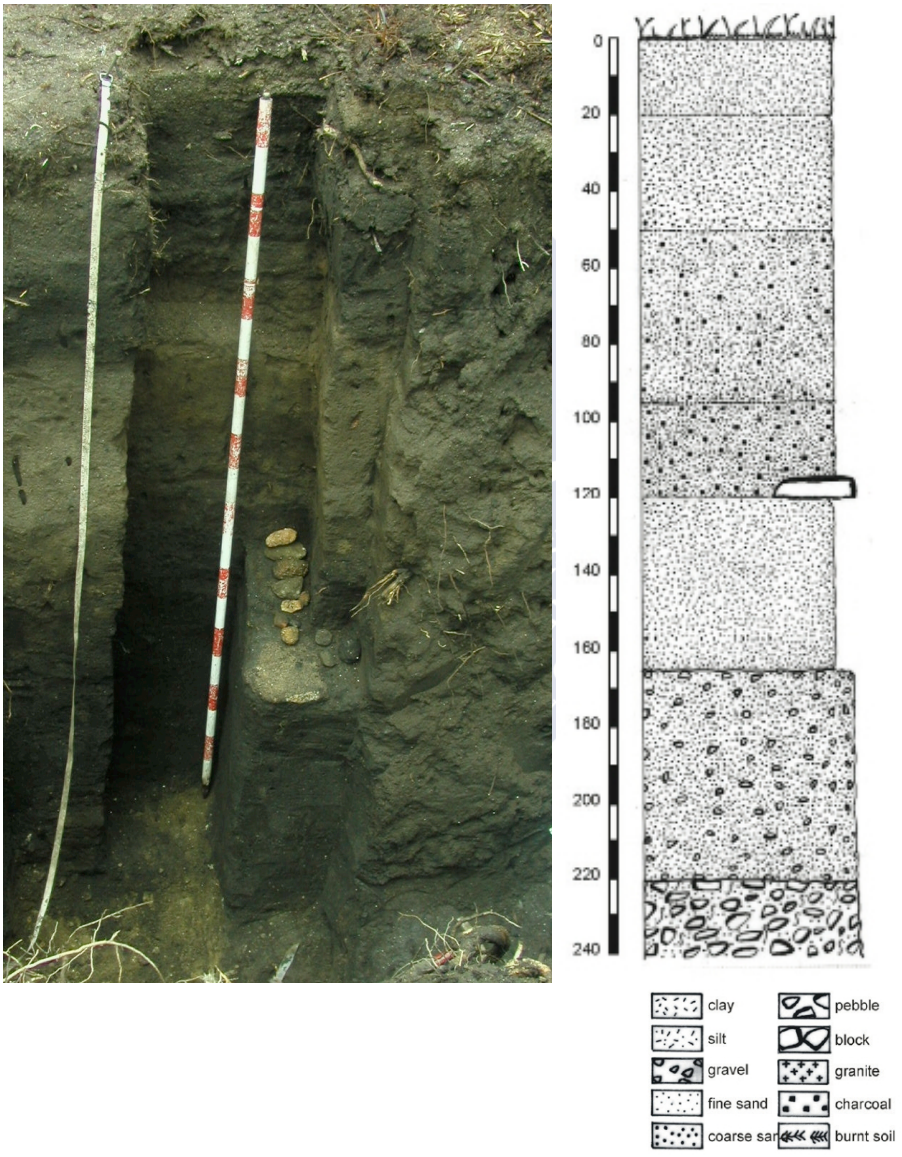

Figure 10. Photograph and profile representation (PRD-4) of a 2.4 m thick haplic umbrisol (humic/alumic) (WRB, 2006) or traditionally referred to as Atlantic ranker from Campo Lameiro (Pontevedra) used for the study in chapter 3. Three samples at different depths were collected: 1) S1 (5-10 cm depth) corresponds to recent material (<150 y BP) that contains considerable amounts of "fresh" SOM; 2) S2 (95-100 cm depth) contains large amounts of charcoal from palaeofires (dated ca. 5 ky ago) and 3) S3 (190-195 cm depth) corresponds to an Early- Holocene phase (ca. 9.7 ky ago) (photograph by courtesy of Joeri Kaal).

are detected, and (iv) the regions from 160 to 220 ppm and from 220 to 245 ppm are assigned to carbonyl C separated into carboxyl/amide and aldehyde/ketone groups, respectively.

2.2.2 Pyrolysis-GC/MS. An introduction to analytical pyrolysis.

Pyrolysis is a chemical degradation/transformation induced by thermal energy –in a range of temperatures between 250 to 800ºC– in absence of any other reagent/catalyst that could be entailed in the process (i.e. performed under inert atmosphere) and that leads to the formation of molecules smaller than the starting material (Moldoveanu, 1998). The originated molecules or so-called pyrolysis products or "pyrolysates" are produced by breaking weak-bonds at a specific temperature of pyrolysis. Pyrolysis cannot identify nor quantify itself. Thus, a joint analysis between pyrolysis and GC/MS (gas chromatography and mass spectroscopy: on-line analysis of volatile pyrolysates) produces and identifies a set of pyrolysis products –termed as "fingerprints"– that gives valuable information from the initial sample (Capelin et al., 1974; Sáiz-Jiménez and de Leeuw, 1986; Moldoveanu, 1998).

2.2.2.1. Pyrolysis-GC/MS analysis description

A brief outline of the Py-GC/MS is described as follows. Solid sample is embedded in glass wool-containing fire-polished quartz tubes and introduced into a Pt filament coil probe on Py-GC/MS. The samples are pyrolyzed usually at range of temperature between 300 to 500ºC (mild pyrolysis) and above 800ºC (vigorous pyrolysis) for 2-5 s with heating rate 10◦C ms. Usually, extracted-SOM is pyrolysed at 600ºC (Nierop et al., 2005; Buurman et al., 2007a) whilst rich-BC samples or coals are subjected to 700-750ºC (Kaal and Rumpel, 2009; Song and Peng, 2010). Thereafter, a flowing of inert-helium (nitrogen or hydrogen are also used) gas moves the fractionated molecules/neo-formed pyrolysates to GC/MS system. The gas chromatographic separation takes places in a column in which the pyrolysates are eluted one by one at different times. The time necessary to elute an injected-component from the chromatographic column is called "retention time" (RT). The retention times of molecules differ because different products move through GC at different rates. The gas chromatographic-eluted pyrolysates are detected in the mass spectrometer and later identified and quantified with

good sensitivity (Moldoveanu, 1998). Once obtained the spectrums, compounds are normally identified using the NIST'05 library and pyrolysis-GC/MS literature (van Smeerdijk & Boon, 1987; Pouwels et al., 1989; Ralph and Hatfield, 1991; Templier and Allard, 2000; Naafs, 2004; Buurman et al., 2007a). Semi-quantification of the relative contributions of the pyrolysis products is based on the surface areas of two characteristic ions (i.e. at m/z 55 + 69 for *n*-alkenes and m/z 57 + 71 for *n*-alkanes). Finally, the combined peak area of all pyrolysis products, the total ion current (TIC) or the so-called *total quantified peak area* (TQPA), is set as 100% and the relative proportions of the pyrolysis products are expressed as the % of the TIC or TQPA.

2.2.2.2. Problems associated to pyrolysis technique

According to several authors pyrolysis is a magnificent tool to provide general information on complex mixtures of compounds (Sáiz-Jiménez and de Leeuw, 1986; Boon and de Leeuw, 1987; Tegelaar et al., 1989; Sáiz-Jiménez, 1994a). However, Sáiz-Jiménez (1994a) stated that this technique is a "double-edge sword", as it can lead to errors if not accompanied by cautious study of the thermal behaviour of the structural units. Indeed, Sáiz-Jiménez (1994a) wrote an article about possible pitfalls, limitations as well as possible solutions that must be carry out during analytical pyrolysis. Kaal (2010) outlines certain issues that must be taken into consideration when using this technique. Firstly, depending on the material and pyrolysis conditions, pyrolysis produces volatiles (pyrolysis products) the fraction of which that is amenable to GC falls within the analytical window but also liquid condensation products (tar) and solid material (char), which are not detected. Secondly, the optimum pyrolysis temperature varies between different types of biomass, i.e. pyrolysis at a given temperature may produce an extensive bond-breaking and also destruction of characteristic chemical moieties in an easily-pyrolysable material whilst in thermally-resistant ones may leave a large residue of non-pyrolysed matter. Third, a set of structural rearrangements, such as elimination of functional groups, cyclisation of linear aliphatics chains, degradation of N-containing functional groups or recombinations may occur during pyrolysis. Fourth, loss of high-polarity compounds in the frequently used non-polar GC column. Fifth, each compound has a different extent of

ionisation resulting in different relative response factors. Besides, some compounds may have been overlooked owing to coelution with more abundant pyrolysis products, and many low-molecular weight compounds have been unnoticed due to elution in the first 2 min of scanning (Kaal et al., 2008). Finally, the presence of mineral particles may catalyse/inhibit some pyrolysis reactions.

2.2.2.3. General chemistry associated to pyrolysis-GC/MS

The heterogeneity and complexity of SOM is based in the mixture of several organic constituents or biopolymers that are derived from plant litter and roots, bacterial biomass, mycelium and fungal structures, tissues from dead animals, animal faeces and their products of decomposition and products of exudation in the case of microbes or plant roots (Stevenson, 1994, Kögel-Knabner, 2002). Plant cell wall components comprise to a large extent the SOM composition although microbial contribution can be also important in determined environments (Naafs, 2004; Nierop et al., 2005). Such components are: (i) cellulose, (ii) non-cellulosic polysaccharides such as hemi-celluloses or pectin, (iii) lignin, (iv) polyphenols (e.g. tannins with attributed antimicrobial properties), (v) lipids, and (vi) cutin and suberin. Biopolymers from intracellular and storage compartments such as (vii) proteins, (viii) starch or (ix) chlorophylls are also valuable in the SOM composition (Kögel-Knabner, 2002).

As result of the pyrolysis assay a wide range of pyrolysates is formed from the molecular breakage of the aforementioned biopolymers (Moldoveanu, 1998). GC/MS provides a wide list of organic compounds that are normally grouped according to chemical similarity and cited as follows: (i) aliphatic compounds (or termed generically as lipids) encompassing to *n*-alkanes/*n*-alkenes, fatty acids, and methyl ketones, (ii) lignin-derived compounds, (iii) the aromatic fraction: monocyclic and polycyclic hydrocarbon compounds, (iv) N-containing compounds, (v) carbohydrate compounds and (vi) other compounds such as sterols, terpenes, etc.

2.2.2.3.a. Aliphatic compounds

Lipids are a heterogeneous group of substances that occur both in plants and microorganisms. They are part of plant cuticle covering the surface of leaves and needles (Kögel-Knabner, 2002). In pyrolysates they encompass *n*-alkanes/*n*-alkenes, *n*-fatty acids and *n*-methyl ketones and are pyrolysis products of cutan, suberan (Tegelaar et al., 1995), cutin, suberin and waxes (Kolattukudy, 1981) and generally they are considered as relatively resistant against degradation (Baldock et al., 1997).

The long-straigh-chain *n*-alkanes and *n*-alkenes are common of non-degradative environments (low microbial activity) or during the first stages of SOM formation, i.e. in fresh litter (Naafs, 2004; Nierop et al., 2005; Buurman et al, 2006). They originate from cuticular waxes of higher plants where odd-chain *n*-alkanes dominate in the C_{25}-C_{35} range (Eglinton and Hamilton, 1967; Sáiz-Jiménez, 1992; van Bergen et al., 1997b, Killops and Killops, 2005), although fresh isolates of such biopolymers may produce a range of *n*-alkanes and *n*-alkenes of chain lengths from below C_{10} to above C_{35} (Eglinton and Hamilton, 1967, Sáiz-Jiménez, 1992; Tegelaar et al., 1995; Almendros et al., 1996; van Bergen et al., 1998a,b; Nierop and Buurman, 1999; Nierop et al., 1999).

n-fatty acids originate from waxes and aliphatic biopolymers such as cutan and suberan, free fatty acids, etc. (Barceló et al., 1992; Tegelaar et al., 1989). Fatty acids found in pyrolysis are largely derived from plants (Tegelaar et al., 1989; Almendros et al., 1996; Chefetz et al., 2002), but they might also be of microbial origin (Amblés et al., 1994; Nierop et al., 2005; Chefetz et al., 2002; Grimalt and Sáiz-Jiménez, 1989; Sáiz-Jiménez, 1996). The *n*-methylketones may be also produced on pyrolysis of fresh plant material (Schellekens et al., 2009), but they are sometimes considered pyrolysis products of microbially-oxidized aliphatic biopolymers (Amblés et al., 1993). Fatty acid methyl esters are pyrolysis products of protective waxes (Killops and Killops, 2005) and quite susceptible to microbial degradation (Buurman and Roscoe, 2010).

Aliphatic compounds are supposed to be rather recalcitrant in soils (Baldock et al., 1997). However, this is only true in soil systems that have a non-ideal decay environment (low pH, low oxygen, low nutrients, low temperature; Buurman and Roscoe, 2010). The long-chain, wax-derived aliphatics are indeed easily degraded in soil and the presence of their pyrolysis products denotes relatively fresh litter (Naafs, 2004). In soil with high-microbial activity (e.g. eutrophic soils with neutral to alkaline pH), degradation process may result in chain-length shortening of aliphatics (Buurman et al., 2007a) although pyrolysis of microbial biomass may also produce short-straigh-chain *n*-alkanes and *n*-alkenes (Buurman et al., 2006). The presence of iso and anti-iso forms of C_{15} fatty acids in top-soils is attributed to bacterial activity (Amblés et al., 1994; Chefetz et al., 2002), but they may also be found in subsoils due to arthropod and fungal activity (Nierop et al., 2005). Short- and mid-chain branched aliphatic pyrolysis products are also associated to presence of charred aliphatic precursors, which would mean that they are indicators of fire activity (Eckmeier and Wiesenberg, 2009; Wiesenberg et al., 2009; Calvelo Pereira et al., 2011). This compendium of likely sources makes difficult their interpretation.

2.2.2.3.b. Lignin-derived compounds

Lignin is an important element of the cell walls of vascular plants, ferns and mosses. After polysaccharides, it is the most abundant biopolymer in nature (Kögel-Knabner, 2002). The primary building units of lignin are the cinnamyl alcohols coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol (Thevenot et al., 2010). Based on differences of monolignol composition we can discern between (i) gymnosperms, composed exclusively of guaiacyl and cinnamyl monomers; (ii) angiosperms, with equal proportions of guaiacyl and syringyl and (iii) lignin of grasses and pine needles with equal proportion of guaiacyl, syringyl and hydroxyphenyl units (Lewis and Yamamoto, 1990, Kögel-Knabner, 2002; Thevenot et al., 2010).

According to some authors, lignin is considered resistant against microbial decomposition (by associations between lignin-cellulose/hemicellulose forming rigid fiber walls) (Kögel-Knabner, 2002; Thevenot et al., 2010) although there is evidence that lignin is

not stable under most pedoclimatic conditions (Lützow et al., 2006) and is highly depolymerised upon decomposition (mainly by white-root-fungi decay). The effect of degradation on oxidation of the C_3 side-chain is easily recognized in pyrolysates. Hence, the relative proportion of moieties with a C_3 side-chain (C_3-guaiacols, C_3-syringols) relative to the total (total guaiacols, total syringols) is frequently used as an index of degradation (C_3G/G_T; C_3S/S_T) (Schellekens et al., 2009). Further degradation (both microbial and/or burning events) of these compounds through demethoxylation produces phenols and methylphenols on pyrolysis (Almendros et al., 2003). However, because phenols and methylphenols are also pyrolysis products of proteins (Chiavari and Galletti, 1992; Galletti and Reeves, 1992; Stuczynski et al., 1997), chitin-protein complexes in arthropods (Stankiewicz et al., 1996; Naafs, 2004), carbohydrates, tannins, suberins, and other biocomponents, their origin remains unknown (Kögel, 1986; van Bergen et al., 1997a, 1998b; Nierop et al., 2001; Nierop et al., 2005). Because the unequal lignin-composition between gymnosperms, angiosperms and grasses, the syringyl/guaiacyl ratio (S/G) or hydroxyphenyl/guaiacyl ratio (H/G) can be used as an estimation of the relative contribution of theses group of plants (Thevenot et al., 2010). Syringyl and cinnamyl units are preferentially degraded compared to the guaiacyl units, resulting in a decrease of the S/G and C/G ratios during lignin degradation (Van der Heijden and Boon, 1994; Nierop et al., 2005). Lignin degradation also promotes increments of the carboxylic acid functional groups compared to aldehyde functional groups (Thevenot et al., 2010). High contents of vinyl-containing phenols and methoxyphenols are also indicatives of fresh lignins (Ralph and Hatfield, 1991).

2.2.2.3.c. Aromatic compounds: monocyclic and polycyclic aromatic compounds

The *monocyclic aromatic compounds* source allocation is generally difficult to establish because they may have multiple origins. For example, phenol and alkylphenols originate from any phenolic precursor including lignin, tannin, proteinaceous biomass, weakly-charred black C and also carbohydrates (Tegelaar et al., 1995; Stuczynski et al, 1997; van Heemst et al., 1999; Kaal et al., 2008), while lignin, tannin or thermally demethylated lignin (Kaal et al., 2012) are the most likely precursors of catechol. Aromatic (e.g. benzene, methylbenzenes,

dimethylbenzenes, ethylbenzenes and styrenes) and also polycyclic aromatic compound (PAH) pyrolysis products may be ascribed to a defined origin only in conjunction with other compounds. In this manner, aromatic pyrolysates are ascribed to proteins (Chiavari and Galletti, 1992), and specifically to microbial material (Schellekens et al., 2009), when pyridine and toluene are found simultaneously, while a combination of toluene and PAHs indicate the presence of charred material (Kaal et al., 2008). Series of *n*-alkylbenzenes (C_4-C_{17}) are attributed to incomplete combustion upon burning (Kaal et al., 2008) but also may be derived from pyrolysis of fresh material.

Polycyclic aromatic hydrocarbon compounds assignment has been subjected to considerable examination. Once considered as evidence of condensation reactions during humification (Schulten et al., 1991), they have now been shown to be an analytical artefact of pyrolysis of aliphatic compounds including *n*-fatty acids (through cyclisation), the intensity of which is stimulated by the presence of reactive mineral components (Sáiz-Jiménez, 1994b; 1995; Almendros, 2008). Indeed, alkyl- benzenes and alkyl-naphthalenes are proposed as likely secondary pyrolysis products of straight-chain fatty acids (Czechowski and Marcinkowski, 2006; Kaal et al., 2008). More recently, PAHs and especially the non-alkyl-substituted and >2 ring PAHs (Rumpel et al., 2007), are considered strong indicators but not always as unequivocal markers of the presence of black C in SOM (Kaal and Rumpel, 2009; Song and Peng, 2010).

2.2.2.3.d. N-containing compounds

Pyrolysis-GC/MS tends to underestimate the contribution of N-containing molecules (Chiavari and Galletti, 1992) but they are commonly found and sometimes in large quantities. Amino acids and amino sugars are by far the most important source of N-compounds during pyrolysis (Galletti and Reeves, 1992) although there is insufficient knowledge of their specific sources (Schulten and Schnitzer, 1998; Van Bergen et al, 1998b). According to van Bergen et al. (1998b) N-compounds can be derived from both microbial or plant. The pyridines, pyrroles and indoles may proceed from microbial activity or be predominantly plant-derived (Buurman et al., 2007b) but also they can be potential products of black N (Kaal et al. 2009).

Diketodipyrrole may be derived from both plant and microbial sources. In order to resolve theses uncertainties, the high occurrence of N-compounds with microbial, fresh litter or charring markers may help us to discern the likely source allocation. Chitin is a polymer of N-acetyl-D-glucosamin linked by ß-(1-4)-glycosidic bonds and is determine as the basic unit of the cell walls of fungi and/or arthropod exoskeleton (Kögel-Knabner, 2002). It is considered relatively easy to degrade in soils (Buurman et al., 2007a) but detailed examination of pyrograms allows to obtain chitin markers. Acetamides and diketopiperazine probably pyrolysis products of chitin and chitin-entangled protein, respectively (Stankiewicz et al., 1996) serving as indicators of biological re-assimilated SOM (Gutiérrez et al., 1995). High amounts of chitin-markers in specific soil are ascribed to high activity of fungal and arthropods rather than to intrinsic recalcitrance (Nierop et al., 2005; Nierop and Buurman, 2007) although they can be protected against complete mineralisation by sorptive protection and/or by physical occlusion (Buurman et al., 2007a).

Indicators of N-containing groups in black C such as benzonitrile and methyl-benzonitriles or markers of "black N" (i.e. recalcitrant heteroaromatic N structures, formed by heat-induced transformation of more or less easily degradable plant proteins; Knicker, 2007) such as isoquinoline, phenylpyridine, benzenedicarbonitriles and pyridinecarbonitriles have recently been postulated (Schnitzer et al., 2007; Kaal et al., 2008b; Song and Peng, 2010) given a useful information about fire events.

2.2.2.3.e. Carbohydrate compounds

The polysaccharide cellulose constitutes the major structural component of the cell walls of plants –comprising between 70-75% of their dry weight (Higuchi, 1997)– but also of cell walls of algae and fungi (de Leeuw and Largeau, 1993). It is composed of glucose units (>10000) linked by ß-(1-4)-glycosidic bonds that in aerobic conditions is rapidly depolymerised. Other non-cellulosic polysaccharides such as hemicelluloses, pectin or starch are decomposed by both aerobic and anaerobic bacteria/fungi with higher decomposition rate than that of cellulose (Swift et al., 1979).

The pyrolysis products from carbohydrates can largely originate from fresh material (well-preserved polysaccharides) or have microbial origin (Sáiz-Jiménez and de Leeuw, 1986; Nierop et al., 2005; Gutiérrez et al., 1995; Buurman and Roscoe, 2010). In fresh litter, pyrolysis products from carbohydrates may prevail above rest of compounds (Murayama, 1984; van Bergen et al., 1997a, 1998a). Levosugars such as levoglucosan, a pyrolysis product of intact polysaccharide (Stuczynski et al., 1997; Poirier et al., 2005; Nierop et al., 2005), cyclopentenones, dianhydro-α-glucopyranose, pyranones and dianhydrorhamnose are largely associated with (relatively fresh) plant-derived matter (Stuczynski et al., 1997; Poirier et al., 2005; Nierop et al., 2005), especially when found in combination with C_3 lignin and low contributions of N-compounds (Buurman and Roscoe, 2010).

Soils with strengthened microbial/fungal activity may produces high amounts of carbohydrates such as furans and furaldehydes associated to dominance over levosugars in a given pyrolysate (Buurman et al., 2007a; Buurman and Roscoe, 2010, Sáiz-Jiménez and Leeuw, 1986) although it can also be associated to charring (Kaal et al., 2008). Indeed, according to some studies high contribution of acetic acid, methylated and acetylated furans, furanone, and furaldehydes in soils could presumably have a microbial origin (Buurman et al., 2007a). Thermal degradation during pyrolysis causes mainly dehydration, decarboxylation, cyclization, condensation and rearrangement of molecular structure of polysaccharides (González-Pérez et al., 2004) and thermal-induced pyrolysates such as furans are frequently associated to charring carbohydrates (Pastorova et al., 1994; Boon et al., 1994). For example, the charring of cellulose (>350ºC) produced a variety of PAHs upon pyrolysis (Pastorova et al., 1994). On the other hand, cellulose fragments may also be encapsulated by a cover of charring during fire and efficiently protected against oxidative agents (de la Rosa, 2008).

2.2.2.3.f. Other compounds

A set of compounds non-allocated in the aforementioned divisions, some of them unidentified or partially identified, are encompassing in this category. Squalene used to be highly found in pyrograms of fresh litter. It is a dihydrotriterpene derived from a vegetal oil component (Heilbron et al., 1926) but can also be derived from fungi (Killops and Killops,

2005). Others such as abietic acid –a diterpene resin acid (Fries et al., 1987)– a pyrolysis product of pine resin or plant steroids are also frequently detected. The latter are structures with four rings mostly consisting of 27 to 30 C atoms, while microbial steroids (hopanoids) have five rings and up to 35 C atoms. Their identification with pyrolysis-GC/MS is problematic, although quite often a group can be identified. Steroids are part of cell membranes (Killops and Killops, 2005) and are generally easily decomposed in aerobic environments, but well-preserved in peats (Schellekens et al., 2009).

2.2.2.3.g. Pyrogenic compounds: BC

Black-C is termed as the product resulting from incomplete thermal combustion of vegetation or/and fossil fuels (Schmidt et al., 2001). Focussed in soils, BC is considered as a versatile type of organic matter with a high specific surface area and resistance against biological degradation (Skjemstad et al., 1996; Golchin et al., 1997; Schmidt et al., 1999; Skjemstad and Taylor, 1999). According its thermal transformation degree (from a slight to intense charring) charcoal particle is described as a heterogeneous mixture of weakly-to-severely aromatised biomass, trapped combustion products and tar, sometimes comprising an uncharred interior (Kaal et al., 2009). Macromolecular structure of black C is defined as set polycondensed aromatic clusters (roughly 15 rings and larger) interconnected through C–C and C–O–C bonds (e.g. Goldberg, 1985) although this assumption matches better with highly polycondensed structures such as soot, graphite, anthracites, etc. (Kaal, 2010). Black C referred to that produced during vegetation fires should be regarded as a heterogeneous mixture of thermally altered biomolecules, consisting of small aromatic clusters (<6 rings on average) linked predominantly through C–C bonds (Knicker, 2007).

According to the aforementioned thermal-transformation degree of the charred material, pyrolysates can largely differ. Weakly charred material or recent charcoal –often with an uncharred core– displays typical pyrolysates of fresh material such as levoglucosan (intact polysaccharides) (de la Rosa et al., 2008) and lignin markers (Rumpel et al., 2007; Kaal et al., 2009) likely derived from sheltered lignocellulose (Knicker et al., 2005b). Besides contributions of furans, short-chain fatty acids and *n*-alkanes/*n*-alkenes, phenols, pyrroles,

pyridines and indole are found together with pyrolysis products of condensed black C such as benzene, toluene and naphthalene (Kaal et al., 2009).

In strongly charred charcoals, black C markers are mainly attributed to benzene and PAHs such as anthracene, napthatlenes, pyrene and biphenyl (Naafs, 2004; Kaal et al., 2009) but by themselves are not indicators of presence of black C because they can have multiple origins. Nonetheless, large contribution of benzenes to the pyrograms in general is a first indication of the presence of significant amounts of BC (Naafs, 2004; Kaal et al., 2008). Thermal treatment or fire results in an enrichment of N-compounds (González-Pérez et al., 2004). Pyrolysis of residues of burnt non-woody material produces high amounts of aromatic N-compounds (Knicker et al., 2005b). N-containing products of BC-rich samples include quinoline, indole, pyridines, pyrroles and benzonitrile (Naafs, 2004; Chefetz et al., 2002) although they cannot be unequivocally used because they are also pyrolysis products of fresh material. N-containing groups in black C or the so-called "black N" (i.e. recalcitrant heteroaromatic N structures, formed by heat-induced transformation of more or less easily degradable plant proteins; Knicker, 2007) such as benzonitrile and methyl-benzonitriles isoquinoline, phenylpyridine, benzenedicarbonitriles and pyridinecarbonitriles have recently been postulated as likely charring indicators (Schnitzer et al., 2007; Kaal et al., 2008; Song and Peng, 2010). The nitriles may have formed upon e.g. dehydration of aromatic amides or amines (Almendros et al., 2003). Aromatic nitriles (i.e. benzonitrile, benzenedicarbonitriles, etc.) may be originated from relatively thermostable/condensed BC although sometimes is related with microbial degradation (Kaal et al., 2009). Finally, the impact of charring on OM chemistry may be examined using a set of ratios (benzene/alkyl-benzenes and PAH/alkyl-PAHs ratios) that provides evidence about degree of dealkylation of aromatic promoted during charring (Kaal and Rumpel, 2009) with subsequent increase of ratios.

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DE COMPOSTELA

3. Aim and outline of this thesis.

The objective of the research presented here was to deepen the understanding of protection mechanisms implied in the SOM stabilization of (i) acid soils that differ in their degree of SOM saturation with Al, (ii) highly-reduced hydromorphic soils; and (iii) those implied in the susceptibility of SOM to chemical oxidation. The persistence of SOM is largely due to complex interactions between SOM, intrinsic soil properties (e.g. the intrinsic compound chemistry, reactive mineral surfaces) and environmental conditions (e.g. temperature, water availability, soil acidity, soil redox state), which influence the presence and activity of microbial decomposers in the immediate microenvironment. With the aim of covering a wide array of (i) SOM types, (ii) intrinsic soil properties and (iii) environmental interactions, a set of variable-charge soils such as andosols, regosols, umbrisols and fluvisols that cover a wide range of pH and redox conditions, mineralogy, water saturation, climate, vegetation were selected in this study. All selected soils are characterized by storing and/or stabilizing high amounts of OC through different organo-mineral interactions.

The oxidant capacity of chemical reagents such as $K_2Cr_2O_7$ and $KMnO_4$ has been investigated and the fraction resistant/susceptible to oxidation established, thus allowing a better understanding of SOM stability based on a chemical-oxidation fractionation. To prove evidences of the aforementioned SOM stabilization/resistance of the studied soils, analytical pyrolysis and solid-state ^{13}C NMR were employed to allow determine the chemical composition of the SOM resistant to oxidation.

The main subjects addressed in this thesis are:

(i) Deepen the understanding of the chemical composition of SOM of acid soils differing in the presence of reactive Al in order to discriminate preferential stabilization mechanisms of SOM.

(ii) The study of the SOM susceptibility to oxidation by chemical reagents of different reactivity and determinate the easily oxidized fractions and/or the resistant fractions.

(iii) To study the biochemistry processes implied in the SOM stabilization in a semiarid mangrove system affected by contaminated rich-nutrient effluents and determine the OC content stocked in mangroves of the study area.

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CHAPTER 2

Comparing NaOH-extractable organic matter of acid forest soils that differ in their pedogenic trend: a pyrolysis GC/MS study

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List of authors:

M. SUÁREZ ABELENDA[a], P. BUURMAN[b], M. CAMPS ARBESTAIN[c,d], J. KAAL[e], A. MARTÍNEZ-CORTIZAS[a], N. GARTZIA-BENGOETXEA[c] and F. MACÍAS[a]

[a]Departamento de Edafoloxía e Química Agrícola, Facultade de Bioloxía, Universidade de Santiago de Compostela, 15782- Santiago de Compostela, Spain

[b]Earth System Science and Climate Change Group, Department of Environmental Sciences, Wageningen University, PO Box 47, Wageningen 6700 AA, The Netherlands

[c]NEIKER-Instituto Vasco de Investigacion y Desarrollo Agrario, Berreaga kalea 1, 48160-Derio, Bizkaia, Spain

[d]Present address: Institute of Natural Resources, Private Bag 11222, Massey University, Palmerston North 4442 New Zealand

[e]Instituto de Ciencias del Patrimonio (Incipit), Consejo Superior de Investigaciones Científicas (CSIC), Rúa San Roque 2, 15704 Santiago de Compostela, Spain

Comparing NaOH-extractable organic matter of acid forest soils that differ in their pedogenic trend: a pyrolysis GC/MS study

Summary

Soil organic matter (SOM) in alu-andic andosols and alu-humic umbrisols is believed to accumulate because of the protection caused by binding to aluminium (Al). We investigated soils that differed in the abundance of organo-Al complexes to determine the effect of such binding on SOM chemistry. For this, the surface horizons of three types of acid soils in the Basque Country (N Spain) under forest stands were studied: (i) alu-andic andosols (AND soils) on basalts and trachytes, (ii) umbrisols or so-called 'aluminic' (ALU) also on basalts and trachytes and (iii) soils with a podzolizing trend (POD), on quartzites. Values of Al extractable with sodium pyrophosphate (Al_p) in the surface horizons of these soils ranged between 8.5-13.1, 1.9-9.3, and 0.8-3.7 g kg^{-1} dry weight, for the AND, ALU and POD soils, respectively. For POD and ALU soils, surface horizons were sampled at two depths: 0-5 and 5-20 cm, whereas the AND soils were sampled at different depths down to the B horizon. NaOH-extractable SOM from three AND soils, 12 ALU soils and 12 POD soils was studied by pyrolysis-gas chromatography/mass spectrometry. The POD soils had the largest loads in plant-derived markers (lignin, long-chain alkanes and alkenes, methyl ketones, fatty acids); SOM of the AND soils had the smallest amounts of plant-derived SOM and the largest amounts of microbial products (microbial sugars and N-compounds) of the soils studied. ALU soils had an intermediate pattern, as expected. The results indicate that the SOM of alu-andic andosols, developed from basalt and trachyte rocks, is essentially dissimilar from that of soils derived from quartz-rich parent material, under the same climate conditions and similar forest stands. The dominance of secondary (microbial-derived) SOM in alu-andic andosols, also observed in previous research on sil-andic andosols (these are dominated by short-range ordered Si compounds in contrast to the dominance of organo-Al complexes in alu-andic andosols) reveals the small contribution of primary (plant-derived) material to SOM in soils with andic properties.

1. Introduction

In temperate humid climates, pedogenesis of alu-andic andosols, alu-humic umbrisols and podzols is, to a large extent, influenced by the weatherability of minerals in the parent material (Macías et al., 2008). Incipient weathering of volcanic material and also of non-volcanic basic and metabasic materials, under humid temperate conditions gives rise to the release of significant amounts of aluminium (Al) and base cations, leading to the accumulation of soil organic matter (SOM) through the formation of organo-Al complexes and the association of organic compounds with short-range order minerals. In these soils, most of the Al released from primary minerals is found in the solid phase, whereas small amounts remain in solution or at exchange sites (Dahlgren et al., 1993). These soils often exhibit andic soil properties and have a typically low organic acidity (Macías et al., 1978; García-Rodeja et al., 1987). In contrast, high organic acidity and large amounts of Al, both in solution and at exchange sites, prevail in surface horizons of forest soils developed from rocks with fewer weatherable minerals. The organic acidity tends to increase as the amounts of weatherable minerals in the parent material decrease, leading to soils in which podzolization processes are dominant (Lundström et al., 2000). Intermediate situations are found in soils developed from parent material of intermediate weatherability or when andic soil properties are not fully developed such as andic dystrudepts (Soil Taxonomy, 2006).

Although the mechanisms for SOM preservation differ widely because of dissimilarities in the prevailing pedogenic processes, all these soils have large SOM contents. The presence of short-range ordered compounds and organo-Al complexes in Andosols has for a long time been considered to protect SOM from microbial attack (Jacquin et al., 1978; Baldock & Skjemstad, 2000). However, recent evidence from studies of allophanic andosols by pyrolysis-GC/MS indicate the absence of a specific preservation or stabilization mechanism for primary SOM, but preferential protection of microbial degradation products by the mineral components characteristic of these soils (Buurman et al., 2007a). Because of the efficient decay of primary (plant-derived) organic detritus, SOM of microbial origin predominates in these soils (Nierop et al., 2005; Buurman et al., 2007a). Buurman et al. (2007a) and Scheel et al. (2008) ascribe the

stability of this SOM to entrapment within the micro-aggregates typical of these allophane-rich soils. Conversely, the chemistry of SOM in the surface horizons of podzols is strongly influenced by the slow decay of fresh organic detritus. Studies using pyrolysis-GC/MS show the dominance of lignin moieties and polysaccharides in the surface horizons, especially in the upper part of the A horizons (Buurman et al., 2007b): there is an important presence of levoglucosan from relatively intact polysaccharides and a small contribution of microbial carbohydrate products. In this study, we hypothesize that SOM of soils dominated by organo-Al complexes is primary of microbial origin, as found in allophanic andosols (Nierop & Jansen, 2009). For this, we investigated the SOM of soils that differed in the abundance of organo-Al complexes, and specifically the surface horizons of alu-andic andosols, umbrisols, incipient podzols under forest stands, located in a small geographical area (the Eastern Cantabrian Range in North Spain). These soils developed on either volcanic or quartzitic parent material, allowing us to assess the effect of Al-saturation on SOM degradation/preservation dynamics. For these purposes, we applied pyrolysis-GC/MS, is an efficient technique in the study of pedogenetic processes. Pyrolysis-GC/MS facilitates the identification of microbial and plant-derived biomass and provides information on the degree of the degradation/preservation of plant debris (Schellekens et al., 2009; Vancampenhout et al., 2009) that are expected to be key characteristics for the study of the molecular characteristics of the SOM of these soils (Naafs, 2004; Nierop et al., 2005; González-Pérez et al., 2007 and Buurman et al., 2007a in allophanic Andosols; Buurman et al., 2007b in Podzols).

2. Materials and methods

2.1. Site and soil description

Two study areas were selected: (i) Moint Oiz, a quartzitic massif and (ii) Moint Karakate, a volcanic massif with dominant basalt and trachyte, both located in the eastern Cantabrian Range (Basque Country, Spain), and 20 km apart (Figure 1). This area has a warm-temperate climate with a mean annual temperature of 13º C and annual precipitation of 1200-1400 mm. Soils were grouped according to the extent of formation and accumulation of organo-Al complexes in the surface horizons. Soils with small amounts of organo-Al

complexes were found in the quartzitic materials, with mean Al_p (Al extractable with sodium pyrophosphate) values ranging from 0.8 to 3.7 g kg^{-1} dry weight and mean $Al_{ox}+1/2Fe_{ox}$ values (the index used for andosol classification) ranging from 2.2 to 7.4 g kg^{-1} dry weight (Table 1): andosols contain >20 g kg^{-1} dry weight. These soils are classified as haplic regosols (dystric) (WRB, 2006) or typic udorthent (USDA, 2006) and will be collectively termed as POD soils.

Soils with the largest amounts of organo-Al complexes were found in the volcanic massif, with Al_p values ranging from 8.5 to 13.1 g kg^{-1} dry weight and $Al_{ox}+1/2Fe_{ox}$ values ranging from 18.7 to 26.6 g kg^{-1} dry weight. These soils are classified as alu-andic andosols (dystric, thixotropic) (WRB, 2006) or pachic fulvudand and pachic melanudand (USDA, 2006) and will be referred to as AND soils. Soils with intermediate amounts of organo-Al complexes were also found in the Karakate massif and have Al_p values ranging from 1.9 to 9.3 g kg^{-1} dry weight and $Al_{ox}+1/2\ Fe_{ox}$ values ranging from 7.7 to 19.2 g kg^{-1} dry weight. These soils are classified as cambic umbrisols (alu-humic), haplic or leptic, depending on thickness, and umbric leptosol (humic, dystric) (WRB, 2006) or andic, humic and typic dystrudept (USDA, 2006). These soils will be referred to as aluminic soils or ALU soils.

For the POD and ALU soils, surface horizons were sampled at 0-5 and 5-20 cm (and identified as upper-A horizon and mid-A horizon, respectively). The surface horizons of the AND soils were sampled down to the B horizon, and subdivided according to their morphological features into two subhorizons, whose depths varied depending on the soil profile: the upper/mid-A horizon was sampled down to 5-30 cm, and the lower-A horizon from 20/30 to 100/135 cm. All soils were under forest stands; POD and ALU soils were under *Pinus radiata* D. Don, whereas the AND soils of the Karakate massif were under oak (*Quercus robur* L.), beech (*Fagus sylvatica* L.), *Podocarpus spp.* and *Chamaecyparis spp.* stands. In order to determinate the influence of vegetation on the studied soils, branches and leaves of the dominant spices (pine, oak and beech) were also analysed. The relevant soil properties are found in Table 1.

2.2 Chemical analysis

The total carbon and nitrogen contents of the soil samples were determined using a TruSpec CHNS analyser (LECO Corp. St. Joseph, MI). Separate acid ammonium oxalate- (Blakemore, 1978) and sodium pyrophosphate- (Bascomb, 1968) extractable Fe (Fe_{ox} and Fe_p, respectively) and Al (Al_{ox}, Al_p) were measured for each sample. Aluminium, Fe and Si were determined by atomic absorption spectrophotometry (Perkin Elmer 2380, Norwalk, CT). Carbon in the sodium pyrophosphate extract (C_p) was determined with a TOC-5000 analyser (Shimadzu Corp., Tokyo, Japan).

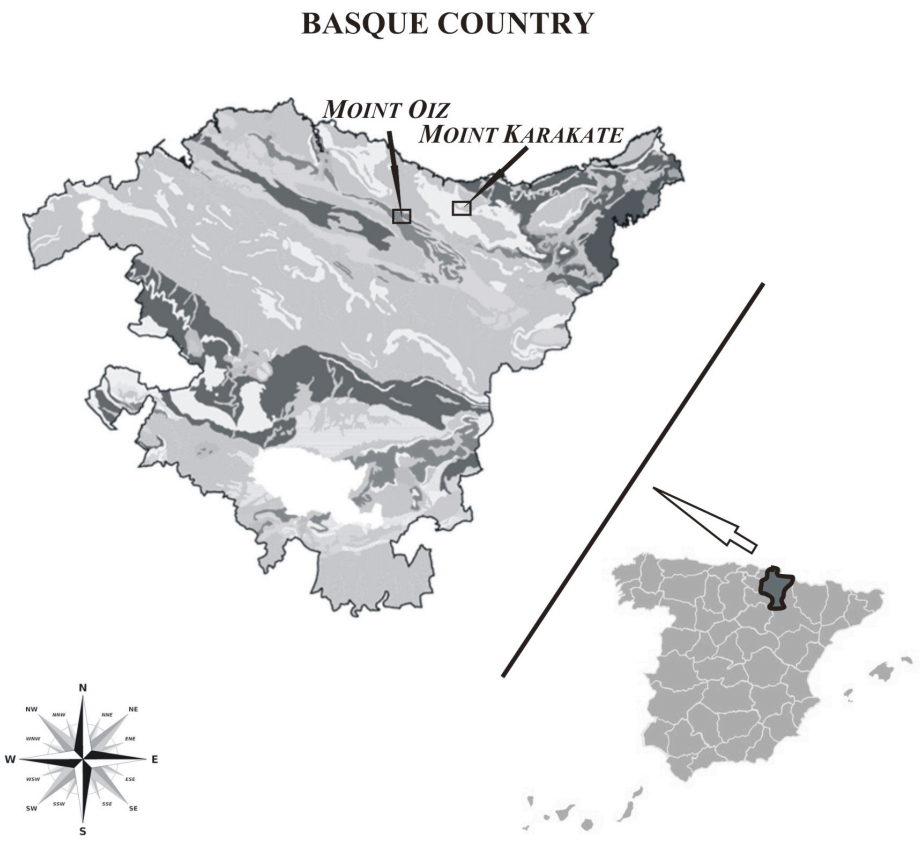

Figure 1. Location of the study area for the soils with a podzolizing trend (Moint Oiz) and Alu-andic Andosols and Umbrisols (Moint Karakate).

2.3 Extraction and purification of SOM

Five grams of air dried soil sample (<2 mm) was extracted with 50 ml of 1 M NaOH and shaken for 24 hours under an N_2 atmosphere to prevent oxidation. The suspension was centrifuged at 2600 *g* for 1 hour and the extract decanted, after which the extraction was repeated. The two extracts were combined and the residue was discarded. The extracts were then acidified to pH 1 with concentrated HCl to protonate SOM. One millilitre of concentrated HF was added to dissolve silicates and increase the content of organic C of the extracted fraction. The acid mixture was shaken for 48 hours, after which it was dialyzed to neutral pH against distilled water to remove excess salt using dialysis membranes with a pore diameter of 6000-8000 Da. Finally, the solution was freeze-dried. Plant samples were finely crushed and analysed without further treatment. It should be noted that, as found in analogous investigations (Naafs, 2004; Nierop et al., 2005; González-Pérez et al., 2007 and Buurman et al., 2007a), not all the SOM was extracted with the NaOH solution, but the extracted fraction is adequate to investigate differences between the soils (data not shown).

2.4. Pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS)

Each purified sample was introduced into a Pt filament coil probe pyrolysis-GC/MS and analyzed with a Pyroprobe 5000 (Chemical Data Systems, Oxford, USA) coupled to a 6890N GC and 5975B MSD GC/MS system from Agilent Technologies (Palo Alto, USA). Approximately 1 mg of the sample was embedded in glass wool-containing fire-polished quartz tubes. Methylstyrene-based contamination was removed from the wool by repetitive heating to 1200° C in the pyrolysis chamber. The samples were pyrolyzed at 650° C for two seconds (heating rate 10° C ms). However, the quartz tubes shielded some of the heat applied to the coil probe from the sample, so that the actual heat applied to the sample was somewhat less than 650° C. Samples were pyrolyzed shortly after insertion into the probe's interface to minimize thermal desorption and degradation before pyrolysis. The interface and GC inlet were set at 325°C and 320°C, respectively. The oven of the GC was heated from 40 to 320°C at 7° C per minute and held at 320^{o} C for ten minutes. The GC/MS transfer line was held at 320° C, the ion source (electron impact mode, 70 eV) at 230°C and the quadrupole detector at 150° C,

Table 1. Selected soil properties of POD, ALU soils (n=5), and AND soils

Code/hz	pH H_2O (1:2.5)		pH KCl		pH NaF		C total (%)		C/N		Al_p		Al_{ox}		Fe_{ox}		Fe_p		Si_{ox}		$Al_{ox}+1/2Fe_{ox}$	
											g kg^{-1}											
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
POD upper-A-hz	4.7	0.5	3.9	0.5	8.6	0.5	5.8	2.1	17.5	1.6	1.9	0.9	1.9	0.8	4.9	1.7	5.0	2.3	0.5	0.1	4.3	1.6
POD mid-A-hz	4.9	0.5	3.9	0.4	9.3	0.3	2.4	1.0	14.4	1.4	2.1	1.0	2.0	1.0	4.6	2.0	5.6	2.5	0.5	0.1	4.3	2.0
ALU upper-A-hz	4.6	0.4	4.0	0.5	8.8	0.6	12.6	4.0	14.5	1.8	6.1	1.9	8.1	2.6	9.2	3.7	6.2	3.5	0.8	0.3	12.3	3.3
ALU mid-A-hz	4.7	0.3	3.9	0.2	9.5	0.5	9.2	3.4	13.6	1.6	7.1	2.4	8.4	1.6	9.0	2.7	6.0	2.4	0.8	0.2	12.9	2.1
AND upper-A-hz	4.5	0.3	3.9	0.1	9.9	0.5	11.0	3.2	16.3	4.2	10.5	1.9	13.1	3.0	16.5	4.6	10.2	2.2	0.7	0.6	21.3	5.2
AND mid-A-hz	4.5	0.1	4.1	0.0	10.9	0.2	5.9	2.1	15.3	4.2	11.3	2.5	12.9	0.8	17.1	5.0	10.7	0.1	0.8	0.0	21.4	3.2
AND lower-A-hz	4.6	0.2	4.2	0.2	10.9	0.5	5.0	3.3	15.3	2.6	11.9	1.0	14.4	1.2	15.3	4.9	9.8	2.9	1.6	0.4	22.1	3.3

These include (i) pH measured in H_2O, KCl and NaF, (ii) total C (Ctotal) and C/N ratio; Al and Fe extractable with pyrophosphate (Al_p; Fe_p); and (iii) Al, Fe and Si extractable with ammonium oxalate (Al_{ox}; Fe_{ox}).

measuring fragments in the *m*/*z* 45-500 Da range. The GC used was equipped with a (non-polar) HP-5MS 5% phenyl, 95% dimethylpolysiloxane column (length 30 m; internal diameter 0.25 mm; film thickness 0.25 µm). Helium was used as the carrier gas (constant gas flow, 1 ml per minute).

Compounds were identified using the NIST '05 library and pyrolysis-GC/MS literature (van Smeerdijk & Boon, 1987; Pouwels et al., 1989; Templier et al., 2000; Ralph & Hatfield, 1991; Naafs, 2004; Buurman et al., 2007a). Semi-quantification of the relative contributions of the pyrolysis products was based on the surface areas of two characteristic ions at *m*/*z* 55+69 for *n*-alkenes and *m*/*z* 57+71 for *n*-alkanes and using MassLab 1.2.7 software. All quantifications were checked. The combined peak area of all pyrolysis products, the 'total ion current' (TIC), was set as 100% and the relative proportions of the pyrolysis products are expressed as the % of the TIC. Note that this is a semi-quantification which is useful to compare samples but, for several reasons, is not equivalent to weight.

2.5. Statistical Analysis

The relative proportions of pyrolysis products were subject to factor analysis using Statistica Version 6 (Statsoft, Tulsa OK, USA). During interpretation of factor analysis components with loadings of less than 0.5 on both factors will not be discussed because less than 25% of their variance is explained by a given principal component.

3. Results and discussion

3.1. General Chemistry

A total of 221 pyrolysis products were used for quantification. These pyrolysis products and their characteristic ions, molecular weight and average retention time (with respect to guaiacol) are listed in Appendix A. To structure the discussion, the compounds were grouped according to chemical similarity into (i) *n*-alkanes/*n*-alkenes, fatty acids, and sterols, (ii) carbohydrates, (iii) lignin, (iv) N-compounds and (v) aromatic compounds (benzenes,

methylbenzenes, dimethylbenzenes, ethylbenzenes and styrenes) and polycyclic aromatic hydrocarbons (PAHs).

3.2. Aliphatics, fatty acids and sterols

Fifty linear aliphatic compounds were identified with a chain length that ranged between C_{10} and C_{33} (Appendix A). In this study, aliphatic compounds refer to *n*-alkanes, *n*-alkenes, *n*-methylketones and *n*-alcohols. The largest contribution of aliphatic components was detected in the AND soils (5.2 and 6.2% of TIC in the H and top/mid A horizons, respectively). Contributions in the ALU and POD ranged from 3.3 to 4.4% in the upper-A horizons and from 3.9 to 4.0% in the mid-A horizons.

The chain length distribution of the *n*-alkanes/*n*-alkenes varied strongly between the different parent materials. In POD soils, long-chain odd-numbered *n*-alkanes (mostly C_{31} but also C_{29} and C_{27}) were dominant. The most abundant *n*-alkene was C_{25}, followed by C_{23} and C_{15}. The long-chain *n*-alkanes and *n*-alkenes are common in fresh litter and in soils with low microbial activity (Naafs, 2004; Nierop et al., 2005; Buurman et al., 2006). These compounds originate from cuticular waxes of higher plants where odd-chain *n*-alkanes dominate in the C_{25}-C_{35} range (Eglinton & Hamilton, 1967; Sáiz-Jiménez, 1992), although fresh isolates of such biopolymers may produce a range of *n*-alkanes and *n*-alkenes of chain lengths from smaller than C_{10} to larger than C_{35} (Eglinton & Hamilton, 1967, Sáiz-Jiménez, 1992; Buurman, 2007a). In soils on volcanic materials (AND and ALU soils), short odd-chain *n*-alkanes and *n*-alkenes were dominant, indicating a more advanced degradation state of the plant debris. Microbial degradation may result in chain-length shortening, but short-chain products may also be of microbial origin (Buurman et al., 2006).

Fifteen *n*-fatty acids were identified in the soils under study (Appendix A). Fatty acids found in pyrolysis are largely derived from plants (Chefetz et al., 2002), but they can also be of microbial origin (Chefetz et al., 2002; Nierop et al., 2005). Fatty acids were scarce in AND soils, with values ranging between 0.3 to 0.8% of TIC, whereas these products were more abundant in POD and ALU soils, ranging between 1.6 and 2.3% of TIC. The small

abundances of fatty acids in AND soils are in agreement with those reported by Naafs (2004) and Buurman et al. (2007a) in allophanic Andosols.

Plant sterols are structures with four rings mostly consisting of 27-30 C atoms, while microbial sterols (hopanoids) have five rings and up to 35 C atoms. Their identification with pyrolysis-GC/MS is problematic, although quite often a group can be identified. Sterols are part of cell membranes (Killops & Killops, 2005) and are generally easily decomposed in aerobic environments, but well-preserved in peats (Schellekens et al., 2009). The presence of sterols in SOM thus suggests the existence of rather fresh material. Their presence is discussed in the factor loadings section.

3.3 Carbohydrates

Pyrolysis products of carbohydrates ranged between 33% in ALU upper-A horizons and 55% in POD lower-A horizons (Table 3). These can have a plant or microbial origin (Sáiz-Jiménez & de Leeuw, 1986; Nierop et al., 2005). Levosugars (Ps18, 23, 24; Appendix A) and cyclopentenones (Ps13, 14; Appendix A) were concentrated in the upper-A horizons (19, 14 and 10% of the total carbohydrate component in POD, ALU and AND soils, respectively). These are largely associated with (relatively fresh) plant-derived matter (Stuczynski et al., 1997; Poirier et al., 2005), especially when found in combination with C_3 lignin and small contributions of N-compounds (Buurman & Roscoe, 2011).

In many soils furans and furaldehydes are derived from microbial biomass, especially when they dominate over levosugars in a given pyrolysate (Sáiz-Jiménez & de Leeuw, 1986; Buurman et al., 2007a; Buurman & Roscoe, 2011). Buurman et al. (2007a) found that the carbohydrate compounds Ps1, 2, 3, 4, 6, 7, 9, 12 and 15 (Appendix A) are well represented in allophanic soils and presumably have a microbial origin. In the present study, carbohydrates had the largest content in the AND soils, with values between 74 and 81% and between 70-73% and 73-76% in the POD and ALU soils, respectively.

3.4 Lignin

In this study, 27 lignin-derived products were identified, with an average abundance of 0.1 to 8.2% of TIC (Appendix A). The largest values were always found in the upper-A horizons, in the order POD > ALU > AND (Table 2). Lignin is the second most abundant component of cellular walls after polysaccharides. It is a polymer of one or more of coumaryl, coniferyl and sinapyl alcohols. Lignin has sometimes been considered to be rather recalcitrant in soils (Buurman et al., 2007a); however, there is now evidence that lignin is not stable under most pedoclimatic conditions (Lützow et al., 2006). Lignin degradation includes oxidation of the C_3 side-chain, which is recognized in pyrolysates. Hence, the relative proportion of moieties with a C_3 side-chain (C_3-guaiacols, C_3-syringols) relative to the total (total guaiacols, total syringols) is frequently used as an index of degradation (C_3G/G_T; C_3S/S_T) (Schellekens et al., 2009). The C_3G/G_T ratios in our soils were always smaller than the C_3S/S_T values, and no clear pattern of these indices was detectable among the three types of soils studied as differences were too small (Table 2).

Gymnosperm lignin consists exclusively of guaiacyl units, whereas angiosperm lignin contains both guaiacyl and syringyl units with a general dominance of syringols (Lewis & Yamamoto, 1990). Therefore, the syringyl/guaiacyl ratio (S/G) can be used as an estimation of the relative contribution of these two plant groups. As expected, for *Pinus radiata* L. plant sample the S/G ratio was close to zero, whereas those of *Fagus sylvatica* L. and *Quercus robur*. L. were close to one (Table 2). The S/G ratios of soil samples ranged between 0.15 and 0.29. This ratio increased with depth in Alu-andic Andosols, which contrasts with the generally supported assumption on the preferential degradation of syringols compared with guaiacols (van der Heijden & Boon, 1994; Nierop et al., 2005). A plausible explanation for this difference is the fact that 150 years ago, before the introduction of exotic species, the native vegetation was dominated by *Fagus sylvatica* L. and *Quercus robur* L. Therefore, the observed depth patterns might reflect this succession of forest species, with a stronger *Pinus* contribution in the upper-A horizons, instead of preferential degradation of specific lignin moieties.

Table 2. Quantified values of different lignin moieties as percentage of the total ion current (% TIC) and decay degree index of soil samples and litter

samples	Total lignin	Sum C_3 guaiacol	Sum guaiacols	C_3G/G_T[a]	Sum C_3 syringol	Sum syringols	C_3S/S_T[a]	S/G[b]	Lg3	Lg5	Lg3+Lg5	Lg3/Lign	Lg5/Lign	Sum vinyl-/ Lign
	TIC	TIC	TIC		TIC	TIC			TIC	TIC	TIC	%	%	%
POD upper-A-hz	28.0	3.5	23.1	0.15	1.1	3.5	0.33	0.15	1.4	3.8	5.1	4.9	13.5	18.4
POD mid-A-hz	13.1	1.2	9.7	0.13	1.0	2.1	0.51	0.21	1.3	2.0	3.3	10.1	15.1	25.2
ALU upper-A-hz	23.4	2.3	18.7	0.12	1.4	3.6	0.40	0.19	1.2	2.2	3.4	5.0	9.6	14.6
ALU mid-A-hz	17.6	1.6	13.8	0.12	0.1	2.7	0.36	0.20	1.0	1.7	2.8	6.0	9.8	15.8
AND H horizon	9.6	0.6	7.1	0.09	0.4	1.5	0.25	0.21	1.0	0.8	1.8	10.8	8.5	19.2
AND upper/mid-A-hz	6.0	0.5	4.3	0.11	0.2	0.8	0.25	0.19	0.8	0.5	1.3	13.8	8.2	22.0
AND lower-A-hz	1.3	0.1	0.8	0.14	0.1	0.2	0.28	0.29	0.2	0.1	0.3	16.5	5.2	21.6
Fagus sylvatica	46.0	6.3	19.4	0.32	6.0	23.3	0.26	1.20	3.3	5.4	8.7	7.1	11.8	19.0
Pinus radiata	40.7	8.3	35.1	0.24	0.2	0.5	0.32	0.01	5.1	10.6	15.7	12.5	26.1	38.6
Quercus robur	35.7	5.6	19.1	0.30	3.6	13.6	0.26	0.71	3.0	4.9	7.9	8.3	13.8	22.1

[a]Index of degradation (C_3G/G_T: C_3 side-chain guaiacols relative to the total guaiacol; C_3S/S_T: C_3 side-chain syringol relative to the total syringol)
[b]syringyl/guaiacyl ratioLg3/Lign: 4-vinylphenol/total lignin ratio; Lg5/Lign: 4-vinylguaiacol/total lignin ratio; Sum vinyl-/Lignin: Sum vinyl-lignin/total lignin ratio

In pyrolysates, the vinyl-containing phenols and methoxyphenols are indicative of fresh lignin (Ralph & Hatfield, 1991). The largest contributions were found in POD soils (25% of lignin contribution), and specifically, at 5-20 cm depth. The greater lignin signal in these POD soils at depth may be explained by recent mechanical reworking of arable layers. In the AND upper/mid horizons contained around 20% total lignin as fresh lignin. The smallest contribution of the markers for fresh lignin was found in the ALU soils (15% of total lignin).

3.5. Aromatic compounds and polycyclic aromatic compounds

In the present study, aromatic compounds were well represented in the AND soils (11.2 to 10.6% of TIC), less abundant in the POD soils (7.0 and 5.2% of TIC) and had intermediate concentrations in the ALU soils (9.9 and 8.2% of TIC) (Table 3). Aromatic (benzene, methylbenzenes, dimethylbenzenes, ethylbenzenes and styrenes) and polycyclic aromatic compounds (PAH) pyrolysis products may be ascribed to a defined origin only in conjunction with other compounds. Thus, aromatic pyrolysates are ascribed to proteins (Chiavari & Galletti, 1992), and specifically to microbial material (Schellekens et al., 2009), when pyridine and toluene are found simultaneously, while a combination of benzene, toluene and PAHs indicate the presence of charred material (Kaal et al., 2008). Values of toluene ranged between 47.3 and 53.8% of total aromatic compounds (Table 3). The largest values of benzene were detected in the AND lower-A horizons (15.9% of aromatic pyrolysates) and the smallest in the POD upper-A horizons (5.6%) (Table 3). Series of *n*-alkylbenzenes (C_4-C_{17}) probably result from incomplete combustion upon burning (Kaal et al., 2008). The AND soils had the largest values of *n*-alkylbenzenes at the surface (14.6% and 14.1% of aromatic fraction in H horizons and upper/mid-A horizons, respectively) and smaller values in the lower-A horizons (6.1% of TIC); values in the POD and ALU soils ranged from 9.6 to 12.5% and 8.5 to 9.6% of aromatic compounds, respectively, values being larger in upper-A horizons than mid-A horizons (Table 3). Polycyclic aromatic hydrocarbons can usually be ascribed to aromatization and condensation reactions upon burning (Kaal et al., 2008). However, their contribution may be under-estimated because of the inefficient pyrolysis of charred residues at the pyrolysis temperature used in this study (650^{o}C). In the pyrolysates under study, PAH compounds were

weakly represented with values ranging from 0.9 to 0.2% of TIC, with the largest value occurring in the AND lower-A horizons (Table 3).

3.6. N-compounds

A large number of N-containing pyrolysis products were identified, of which 30 were quantified (Appendix A). Although N-compounds are, in general, pyrolysis products of amino acids and amino sugars (Galletti & Reeves, 1992), there is insufficient knowledge of their more specific sources (Schulten & Schnitzer, 1998; van Bergen et al., 1998). In this study attempts were not made to identify the specific protein sources, but rather to distinguish between N-compounds from plants and microbial biomass. According to van Bergen et al. (1998), a large number of N-compounds are from microbial SOM, but others can be both of microbial and plant origin. The pyridines and pyrroles that were quantified in the present study are probably of microbial origin, while the indoles can be predominantly plant-derived (Buurman et al., 2007b). The sum of pyridines and pyrroles comprised 67% of the N- compounds in POD soils, and 83% in AND lower-A horizons. Indoles had a larger abundance in quartzitic mid-A horizons (10%) compared with the AND lower-A horizons (3%). Acetamides are probably pyrolysis products of chitin (Stankiewicz et al., 1996). Chitin is a polymer of N-acetylglucosamine (a N-containing polysaccharide) that is relatively easy to degrade (Buurman et al., 2007a), and its pyrolysis products are not commonly found in soils. Nevertheless, a large number of chitin pyrolysis products were obtained from the allophanic soils of the Azores (Nierop et al., 2005; Nierop & Buurman, 2007) and Costa Rica (Buurman et al., 2007a). This was ascribed to high fungal activity rather than to recalcitrance. In the present study, however, pyrolysis products of chitins were scarce (<1.2% in POD mid-A horizons and about 0.6% in AND lower-A horizons). Diketodipyrrole may be derived from both plant and microbial sources. The proportion of this compound was largest in AND soils (2.1-1.1% in litter and at depth, respectively). Several compounds with nitrile functional groups were detected (N17, 18, 29, 30; Appendix A), accounting for 1.6-0.3% of TIC (Table 3). The origin of these compounds remains unknown, but they may be related to charring.

3.7. Factor analysis

Four extracted factors explained 67% of the variation of all pyrolysis products, while Factor 1 and Factor 2 together explained 46%. The following discussion will focus on F1-F2 factor space. Figure 2 contains all quantified variables and shows a clear differentiation of chemical groups in F1-F2 space. Straight-chain C_{13}-C_{24} alkanes form a distinct cluster at the far right in the SE quadrant. The odd-numbered long-chain alkanes (C_{29}-C_{33}) are at the far left. The C_{13}-C_{24} alkenes occur close to the alkane cluster. Some *n*-alkanes, especially the even-numbered long-chain members, are present between the two clusters, which can either be because there is more than one source or because of smaller abundances with a larger deviation. The mid-chain *n*-methylketones all occur at the bottom of the lower right quadrant of Figure 2. This is remarkable, because they usually occur close to the *n*-alkane analogues. The long-chain *n*-methylketones (C_{26}-C_{31}) are close to the long-chain (C_{29}-C_{33}) alkanes. Most fatty acids, excepting F10 and F13 (Appendix A), are in the SW quadrant, with the most abundant, plant-derived ones at the far left. Fatty acid methyl esters have negative loadings on F2.

All lignin products have negative loadings on F2; they occur largely in the SW quadrant. Some syringol compounds (Lg21, 23, 26, 27; Appendix A) and one guaiacol (Lg16; Appendix A) are further to the left. The aromatic compounds occur in or close to the NE quadrant, with benzene (Ar1; Appendix A) towards the top and the other compounds close together. The alkylbenzenes (B4-B17; Appendix A) are largely in the mid-chain *n*-alkane/*n*-alkene cluster, in the SE quadrant. Polycyclic aromatic hydrocarbons, indene (PA1) and naphthalene (PA2 and 3) potentially char-derived (Kaal et al., 2008) and microbial products (Buurman et al., 2007a), spread in the SE quadrant while phenanthrene (PA4) occurs in the SW quadrant; both clusters provide little information in F1 factor space. One of the nitriles (N30; Appendix A) occurs with the PAH compounds.

The N-compounds are spread throughout the F1-F2 factor space, but have some concentration in the NE and NW quadrants. The compounds that are probably microbial are found in the NE quadrant, while those attributable to plant material are found at the far left.

Table 3. Quantified values of detected pyrolysates as percentage of the total ion current (% TIC)

samples	Aliph	Alcanols	Ps total	Ps litter	Ps micr	Ar total	Benzene	alkyl-benzenes	Toluene	PA	FA	methyl esters	Pyridines+ pyrrols	indols	chitin	diketodipyr	Sq	dehydr acid
	% TIC		% TIC	% /Ps		% TIC	% /Ar			% TIC			% /N			% TIC		
POD upper-A-hz (0-5 cm)	3.35	0.10	40.7	19.6	70.3	7	5.6	9.6	47.3	0.17	1.9	0.14	67.4	12.4	0.61	0.9	0.01	0.15
POD mid-A-hz (5-20 cm)	3.96	0.12	54.9	14	73.5	5.2	6.3	12.5	48.8	0.1	2.3	0.14	67.4	10	1.17	0.7	0.03	0.12
ALU upper-A-hz (0-5 cm)	4.39	0.09	33	13.7	73.8	9.9	6.5	8.5	50.6	0.17	2	0.13	69	9.5	0.67	1.8	0.02	0.32
ALU mid-A-hz (5-20 cm)	4.08	0.08	39.6	11.6	76	8.2	6.2	9.6	50.7	0.12	1.6	0.12	70.1	9.1	0.77	2.1	0.01	0.43
AND H hz	5.25	0.09	39.1	13.9	69.4	10.6	6.4	14.6	46.8	0.17	0.8	0.14	72.5	7.1	0.75	2.1	0.03	0.24
AND upper-A-hz (0-5/30 cm)	6.24	0.08	41.1	10	74.4	11.2	8.9	14.1	46.9	0.22	0.5	0.08	73.3	5.7	0.86	1.7	0.04	0.25
AND lower-A-hz (20/30-100/135 cm)	2.75	0.03	50.9	8.5	80.8	10.6	15.9	6.1	53.8	0.07	0.3	0.06	80.3	2.8	0.6	1.1	0.03	0.85
Fagus sylvatica	3.0	0.15	31.4	12.2	71.7	2.9	6.8	7.5	61.6	0.05	3.3	0.24	57.1	26.1	0.07	0.3	0.09	0.00
Pinus radiata	1.6	0.6	29.4	18.9	54	3.8	31.7	7.7	42.2	0.12	5.5	0.2	66.4	16.8	2.65	0.2	0.01	0.22
Quercus robur	2.4	0.34	33.9	16.3	69.4	5.4	9.3	4.8	61.5	0.12	3.8	0.17	54.2	25.6	0.01	0.6	0.09	0.00

Benzene, alkyl-benzenes and toluene are given as percentage of the total aromatic compounds. Indols, chitin and sum of piridines and pyrrols are given as percentage of the nitrogen compounds.

Aliph: aliphatic compounds; Ps litter: polysaccharides derived from litter; Ps microb: microbial polysaccharides; Ar: aromatic compounds; PA: polyaromatic compounds; FA: fatty acids; diketodipyr: diketodipyrrole;Sq: squalene; dehydr acid: dehydroabietic acid.

The microbial N-compounds occur in the same area as toluene (Ar2), which implies that these have a microbial source. The chitin-derived acetamides (N23-25, 28; Appendix A) are in the left hand part of the diagram, with low loadings on F2. Whether from fungi or arthropods, this projection indicates that the chitin is associated with the initial stage of litter decomposition. Sterols, and carbohydrate pyrolysis products that indicate relatively fresh SOM (Ps15, 18, 23, 24; Appendix A) are in the left hand part of the diagram, while furans and cyclopentenones (Ps 1, 7, 8, 9, 10, 13, 16 and 17; Appendix A) are spread throughout the positive F1 factor space and close to N-compounds and away from levosugars. Large amounts of N-compounds accompanied by carbohydrate pyrolysis products other than levosugars point toward the presence of large amounts of microbial SOM (Buurman & Roscoe, 2011). Furan-2-one (Ps4) and 2,3-dihydro-5-methylfuran-2-one (Ps11) occur at top centre between the plant-derived and microbial carbohydrates. All phenols, except for one methoxycatechol (Ph9), occur together with the microbial sugars and N compounds, indicating a proteinaceous origin.

Overall, the location of pyrolysis compounds in the factor space indicates that large negative loadings on F1 are associated with relatively fresh SOM. The NE quadrant is associated with microbial SOM. The concentration of mid-chain aliphatics in the SE quadrant indicates a relatively recalcitrant fraction from which palatable compounds have been stripped and where microbial products have not accumulated. The transition from centre left, via the bottom of the diagram, towards the SE alkane cluster (see arrow in Fig. 2) illustrates a decomposition system with, from left to right, decreasing amounts of palatable compounds. In this process, the following compounds consecutively disappear: (i) long-chain alkanes and methyl-ketones, specific plant-derived alkenes, and steroids; (ii) syringol lignins followed by the remainder of the lignin group; and (iii) esters and methylketones. The placement of the two furanones, at the top centre between the two polysaccharide clusters, suggests that these are decomposition products of plant-derived SOM and that they disappear upon further microbial degradation.

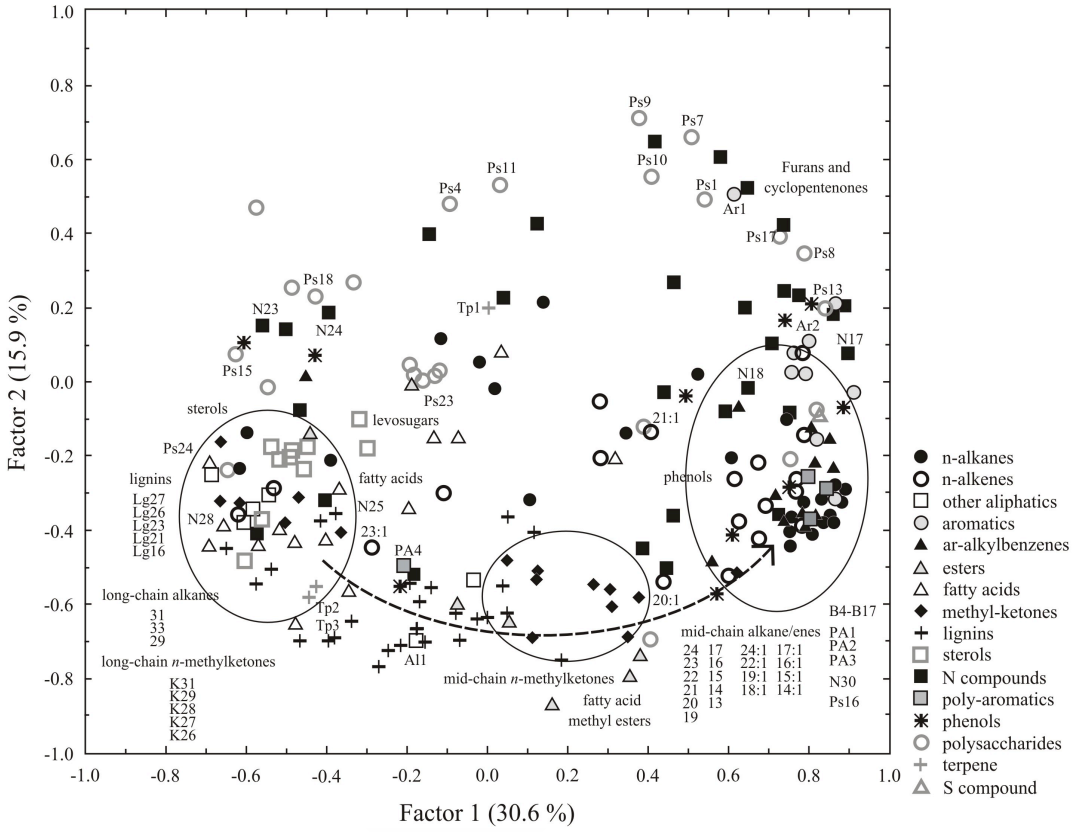

Figure 2. Projection of the factor loadings of the pyrolysis products in the F1-F2 space. Straight-chain alkanes (number) and alkenes (number:1 -one double bond) and other aliphatic compounds (Al), aromatics (Ar), alkylbenzenes, esters, fatty acids (FA), methyl-ketones (K), lignins (Lg), sterols, N-containing compounds (N), poly-aromatics (PA), phenols, polysaccharides (Ps), Terpenes (Tp) and S compound.

Figure 3 shows the plot of sample scores in the F1-F2 space. The most pronounced cluster is that of the AND lower-A horizons at the top centre, with strong positive scores on F2. AND upper/mid A horizons string out from this cluster towards the lower right area. Most of the other samples form a cloud that extends from the centre to the left part of the diagram, with most of the POD mid-A horizons clustering at far left. POD upper-A horizons had more negative scores on F2. The main differentiation is thus between (i) the AND lower-A horizons, (ii) the AND upper/mid A horizons plus some ALU upper-A horizons and POD mid-A horizons under young pine stands and (iii) the POD mid-A horizons under mature pine stands.

The SOM properties underlying the segregation observed in Figure 3 can be derived from the loadings shown in Figure 2.

The combined information from Figures 2 and 3 indicates that the AND lower-A horizons contain the smallest amounts of plant-derived SOM (lignin, alkanes/alkenes, methyl ketones and fatty acids) and the largest amount of microbial products (microbial sugars and proteins). The AND upper/mid-A horizons and some of the ALU upper-A horizons are strung out towards the SE quadrant, which indicates larger amounts of (recalcitrant) aliphatic biopolymers. Both more microbially-degraded SOM (lower A-horizons) and an accumulation of recalcitrant SOM (upper A-horizons) are thus found in soils rich in weatherable minerals and with a dominance of reactive Al compounds, as can be inferred from their Alp and Alox values (Table 1). The POD mid-A horizons under mature pine stands appear to contain the largest amounts of relatively fresh SOM, while the POD upper-A horizons under the young pine stands appear to have lost some carbohydrates, resulting in slightly larger amounts of lignin and alkanes, alkenes and methylketones. These unexpected results can be explained by the fact that some of the POD sites under young pine stands had recently undergone mechanical disturbance, as some plantations were second rotation pine stands at their early stages (first year) (Gartzia-Bengoetxea *et al*., 2010). In the ALU soils, the upper-A horizons appear to contain more lignin and methyl ketones than the mid-A horizons, as expected. The SOM of the ALU soils appears to be more decomposed than that of the corresponding POD soils. Some POD mid-A horizons under young pine stands and ALU upper-A horizons appear to have microbial contributions and decay, but never as extensively as in the AND lower-A horizons. Overall, the results reflect the presence of less decomposed SOM in soils poor in weatherable minerals and with few reactive Al surfaces.

Some differences between our study and some recent studies are worth mentioning. Contrary to what has been found in allophanic soils from the Azores Islands (Nierop et al., 2005), the soils studied here did not provide evidence of large contributions of chitins from fungal and arthropodal SOM and fatty acids from bacteria. Accumulation of SOM in allophanic andosols from Costa Rica (Buurman et al., 2007a) was attributed to the strong

biological activity. In the present soils, however, biological processing seems to be much less intense. This is probably related to the fact that soils from Costa Rica are located in a tropical environment, whereas those from the Basque Country are from a temperate climate. Buurman et al. (2007a) concluded that, in the andosols from Costa Rica, an allophane content greater than 5% appeared to favour the decomposition of plant-derived material. This is not exclusive to allophanic andosols, because we also found a high degree of decomposition in alu-andic andosols.

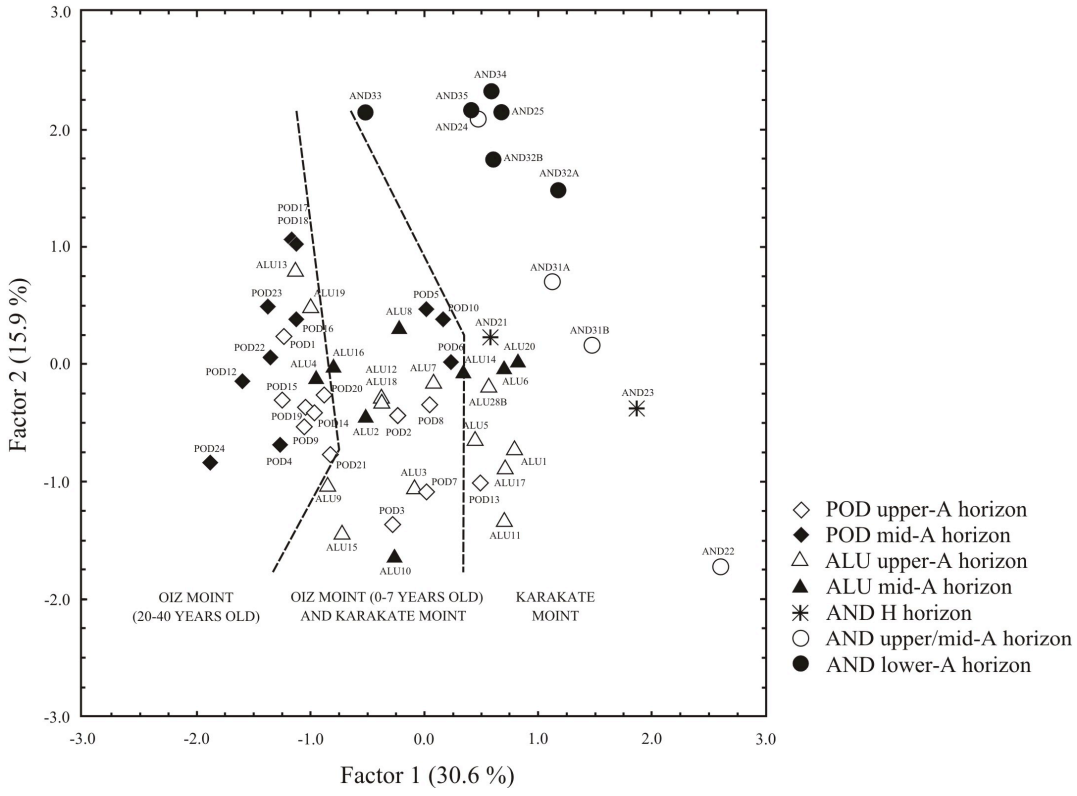

Figure 3. Projection of factor scores in the F1-F2 space, coded by soil type. Diamonds represent samples collected between 0 and 5 cm (white) and 5-20 cm (black) depth of soils with a trend for Podzol development; triangles represent samples collected between 0 and 5 cm (white) and 5-20 cm (black) depth of 'aluminic' soils. Asterisks represent samples from litter layers of andic soils while circles represent samples of upper/mid-A horizon sampled down to 5-30 cm depth (white) and lower-A horizon from 20-30 to 100-135 cm (black) of andic soils. Discontinued lines describe location and growing period.

4. Conclusions

Soils developed on materials poor in weatherable minerals such as quartzites, under humid temperate conditions and good drainage, are acidic and highly dystrophic, and they eventually podzolize. As such, these soil systems do not provide an ideal decay environment (Buurman & Roscoe, 2011), and primary organic matter tends to accumulate at the surface, although a soluble fraction may be mobilized and preferential root growth may occur in the subsurface horizon. This is reflected in rather fresh SOM in the A horizons. As the dystrophic conditions in these soils are accompanied by a lack of reactive Al compounds, we cannot discern whether the presence of these reactive surfaces would have promoted the stabilization of primary organic matter.

Soils developed in materials rich in weatherable minerals such as basalt or amphibolite, under humid temperate conditions and good drainage, are also acidic, but less dystrophic, as these materials are rich in basic cations. To a certain extent, these soil systems thus have an ideal decay environment for fresh organic matter. During decomposition, the acidity released from the primary organic matter is buffered by the alkalinity released by the weathering of primary minerals including ferromagnesian and plagioclase minerals, and Al discharged from the latter interacts with secondary organic matter, forming organo-Al complexes. These Al-SOM precipitates form micro-aggregates that, in addition to the intrinsic stability provided by the precipitation of these organo-Al complexes (in which Al forms two to three bonds to the organic compounds), offer a further stabilization mechanism to SOM against microbial attack, by reducing the access of microbes (Scheel et al., 2008). Unpublished data on aggregate fractionation of the soils under mature forest stands from both massifs showed a dominance of micro-aggregates (50-250 μm) in those developed from volcanic materials, with almost half of the soil aggregates included in this fraction. In soils formed on quartzites, this fraction only represented 23% of the soil aggregates and mega-aggregates (2-20 mm) were dominant. Buurman et al. (2007a) suggested that the anaerobiosis generated within the micro-aggregates formed in allophanic andosols under perudic conditions may further decrease the rate of SOM decomposition. The results obtained here showed that decomposition affected all plant-derived

components, without any evidence of the existence of a specific recalcitrant fraction in primary SOM. Therefore, under the specific pedogenic conditions of andic soils, once primary SOM has been decomposed under the initial ideal conditions, non-ideal conditions emerge for decomposition of secondary SOM.

The results on alu-andic andosols are therefore similar to those obtained in allophanic andosols developed on volcanic deposits: the SOM in the alu-andic andosols studied here is mostly secondary SOM. Overall, the SOM in these alu-andic andosols is essentially different from that in the POD soils developed under similar forest stands but in quartzitic parent material. The latter was found to contain the least decomposed SOM among the soils studied.

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Appendix A. Maximum and minimum relative abundances of individual pyrolysis products are given as the percentage of total quantified peak area (% TIC).

Code	Name	M	m/z	RT-GUAIACOL (min)	% TICsoils (max)	% TICsoils (min)	% TICveg (max)	% TICveg (min)
10:0-33:0	alkanes C10-C33	142-450	57+71	0.785-4.064	0.13-0.98	0.00-0.03	0.00-0.56	0.00-0.05
10:1-28:1	alkanes C10-C28	140-392	55+69	0.767-3.578	0.14-0.95	0.00-0.03	0.02-0.40	0.00-0.03
Al1	C16 alcohol	242	55+57	2.453	0.14	0.00	0.03	0.02
Al2	oxacycloheptadec-8-en-2-one	252	67+81	2.540	0.10	0.00	0.14	0.01
Al3	oxacycloheptadecan-2-one	198?	55+69	2.550	0.24	0.00	0.06	0.00
Al4	9,12,15-nonadecatrien-1-ol	264	67+79	2.901	0.17	0.00	0.57	0.12
Al5	alkene 23aC	n.d.	55+69	2.975	0.24	0.00	0.00	0.00
Al6	branched alkene	n.d.	83+280	3.069	0.32	0.00	0.06	0.01
Ar1	benzene	78	77+78	0.259	2.54	0.00	1.19	0.21
Ar2	toluene	92	91+92	0.363	9.10	0.28	3.98	1.60
Ar3	ethylbenzene	106	91+106	0.510	0.98	0.05	0.31	0.15
Ar4	1,2 dimethylbenzene	106	91+106	0.524	1.03	0.01	0.48	0.22
Ar5	styrene	104	78+104	0.564	1.39	0.08	0.43	0.19
Ar6	1,3 dimethylbenzene	106	91+106	0.569	0.61	0.03	0.00	0.00
Ar7	benzene, 1,3,5-trimethyl-	120	105+120	0.766	0.45	0.02	0.05	0.01
Ar8	ethyl-methylbenzene	120	105+120	0.847	0.61	0.02	0.12	0.05
Ar9	benzene, 1-propenyl	118	117+118	0.848	0.39	0.02	0.10	0.05
B4-B17	C4-C17 benzene	134-316	91+92	0.896-3.147	0.04-0.66	0.00-0.03	0.00-0.15	0.00-0.04
E1	C-16 acid. methyl ester	270	74+87	2.503	0.13	0.01	0.15	0.08
E2	heptadecanoic acid, 2-propenyl	296	100+113	2.725	0.06	0.00	0.03	0.01
E3	C-18 acid. methyl ester	298	74+87	2.791	0.11	0.01	0.02	0.01
E4	octedecanoic acid, 2-propenyl	324	100+113	2.997	0.06	0.00	0.00	0.00
E5	C-20 acid. methyl ester	326	74+87	3.048	0.03	0.00	0.03	0.01
E6	C-22 acid. methyl ester	354	74+87	3.287	0.04	0.00	0.04	0.01
E7	C24 acid, methyl ester	382	74+87	3.501	0.03	0.00	0.02	0.00
F8	FA (C8)	144	60+73	1.238	0.45	0.02	0.41	0.17
F10	FA (C10)	172	60+73	1.602	0.22	0.00	0.18	0.02
F12	FA (C12)	200	60+73	1.959	0.91	0.00	1.09	0.06
F13	FA (C13)	214	60+73	2.130	0.05	0.00	0.04	0.01
F14	FA (C14)	228	60+73	2.279	1.37	0.00	0.55	0.12
F15	FA (C15)	242	60+73	2.430	0.21	0.00	0.02	0.00
F15a	iso FA(C15)	242	60+73	2.385	0.08	0.00	0.01	0.00
F15i	anti-iso FA(C15)	242	60+73	2.397	0.07	0.00	0.05	0.03
F16	FA (C16)	256	60+73	2.585	1.85	0.00	2.75	1.96
Fo	oleic acid	282	55+57	2.652	0.25	0.00	0.02	0.00
F17	FA (C17)	270	60+73	2.704	0.2	0.00	0.04	0.02
F18	FA (C18)	284	60+73	2.850	0.74	0.00	1.09	0.22
F18u	FA18:1	282	55+69	2.821	1.71	0.00	0.15	0.02
F20	FA (C20)	312	60+73	3.106	0.11	0.00	0.13	0.01
F22	FA (22)	340	60+73	3.337	0.05	0.00	0.15	0.01
K15-K31	n-C15:0-C31:0 MK	254-478	58+59	2.161-4.089	0.02-0.10	0.00-0.01	0.00-0.04	0.00-0.01

Appendix A. (continuation)

Code	Name	M	m/z	RT-GUAIACOL (min)	% TICsoils (max)	% TICsoils (min)	% TICveg (max)	% TICveg (min)
Lg1	guaiacol	124	109+124	1.000	17.4	0.12	9.47	4.44
Lg2	4-methylguaiacol	138	123+138	1.221	3.47	0.02	3.43	1.96
Lg3	4-vinylphenol	120	91+120	1.304	2.20	0.14	5.07	2.25
Lg4	4-ethyl, guaiacol	152	137+152	1.400	2.70	0.02	2.78	1.41
Lg5	4-vinylguaiacol	150	135+150	1.477	5.09	0.05	10.64	4.42
Lg6	syringol	154	139+154	1.554	1.96	0.01	5.05	0.07
Lg7	4-(2-propenyl) guaiacol	164	77+164	1.558	0.23	0.00	0.54	0.37
Lg8	cis 4-propyl guaiacol	166	137+166	1.576	0.27	0.00	0.43	0.28
Lg9	4-propylguaiacol trans	164	149+164	1.641	0.10	0.01	0.45	0.30
Lg10	cis guaiacol-4-(1-propenyl)-	164	91+164	1.674	0.17	0.00	0.41	0.29
Lg11	4-methylsyringol	168	153+168	1.733	0.43	0.00	2.44	0.13
Lg12	trans guaiacol-4-(1-propenyl)-	164	149+164	1.738	0.96	0.01	3.08	1.90
Lg13	4-acetylguaiacol	166	151+166	1.826	4.08	0.03	0.65	0.45
Lg14	vanillic acid methyl ester	182	151+182	1.87	1.13	0.00	0.69	0.09
Lg15	4-ethylsyringol	182	167+182	1.879	0.40	0.03	1.55	0.01
Lg16	2-propananone guaiacol	180	137+180	1.903	3.36	0.00	1.92	0.91
Lg17	4-vinylsyringol	180	165+180	1.952	0.50	0.00	5.30	0.03
Lg18	4-propanedione guaiacol	180	151+180	1.987	0.60	0.00	0.20	0.14
Lg19	4-(2-propenyl) syringol	194	91+194	2.005	0.05	0.00	0.89	0.00
Lg20	4-propylsyringol	196	167+196	2.007	0.05	0.00	0.35	0.02
Lg21	vanillic acid	168	153+168	2.054	4.84	0.00	0.00	0.00
Lg22	4(prop-2-enyl) syringol, cis	194	91+194	2.094	0.07	0.00	0.70	0.07
Lg23	dihydroconyferyl alcohol	182	137+182	2.117	0.15	0.00	1.20	0.49
Lg24	4-(prop-2-enyl)syringol, trans	194	91+194	2.184	0.21	0.00	3.97	0.00
Lg25	4-acetylsyringol	196	181+196	2.248	0.6	0.01	0.77	0.01
Lg26	4-(Propan-2-one)syringol	210	167+210	2.301	0.36	0.00	1.46	0.01
Lg27	propenyl syringol	194	70+194	2.572	7.83	0.03	0.35	0.08
N1	N-methylpyrrole	81	80+81	0.332	2.40	0.00	0.81	0.39
N2	pyrrole	67	67	0.350	3.69	0.00	1.56	1.02
N3	pyridine	79	52+79	0.353	8.97	0.61	0.63	0.51
N4	2-methyl pyridine	93	66+93	0.453	1.54	0.04	0.22	0.12
N5	2-pyrimidine. x-methyl	108	107+108	0.458	0.54	0.01	0.23	0.11
N6	C1-pyrrole 1	81	80+81	0.477	2.89	0.00	0.15	0.00
N7	C1-pyrrole 2	81	80+81	0.491	3.21	0.00	0.14	0.04
N8	3-methyl pyridine	93	66+93	0.527	2.03	0.05	0.27	0.18
N9	1H-pyrrole, 3-ethyl	95	80+95	0.638	0.96	0.00	0.00	0.00
N10	pyridine, 2-4-dimethyl	107	107+106	0.660	0.64	0.00	0.00	0.00
N11	4(1H)-pyrimidinone, 6-methyl	110	54+110	0.751	1.46	0.10	0.50	0.36
N12	pyridine, 3-methoxy	109	66+109	0.806	1.57	0.10	0.00	0.00
N13	1H-pyrrole, 2-ethyl-4-methyl	109	94+109	0.815	2.07	0.00	0.00	0.00
N14	benzenamide, 2-methoxy	123	108+123	0.953	0.56	0.01	0.00	0.00

Appendix A. (continuation)

Code	Name	M	m/z	RT-GUAIACOL (min)	% TICsoils (max)	% TICsoils (min)	% TICveg (max)	% TICveg (min)
N15	ethanone, 1-(1H-pyrrol-2-YL)-	109	94+109	0.957	0.65	0.00	0.12	0.03
N16	2,5-pyrrolidinedione, 1-methyl	113	56+113	1.012	0.77	0.00	0.08	0.02
N17	benzeneacetonitrile	117	90+117	1.107	1.13	0.13	0.26	0.11
N18	benzenepropanenitrile	131	91+92	1.321	0.81	0.10	0.65	0.29
N19	indole	117	90+117	1.442	1.71	0.22	1.44	0.47
N20	imidazole, 2-acetamino-5-methyl?	139	97+139	1.498	0.54	0.00	0.00	0.00
N21	1H-indole, 4-methyl	131	130+131	1.624	1.04	0.05	0.81	0.4
N22	pyridine 2-phenyl	155	154+155	1.769	0.28	0.00	0.05	0.04
N23	acetamide, N-(2,4-dihydroxyphenyl)	167	125+167	1.87	1.49	0.19	0.00	0.00
N24	acetamide, N-(3,4-dihydroxyphenyl)	167	69+125	1.912	0.71	0.01	0.00	0.00
N25	alpha-acetamidocinnamic acid	205	117+187	2.117	0.15	0.00	0.03	0.00
N26	diketodipyrrole	186	93+186	2.200	3.42	0.39	0.78	0.18
N27	alkylamide	n.d.	59+72	2.684	0.10	0.00	0.00	0.00
N28	acetamide compound	237	195+237	2.719	0.08	0.00	0.14	0.00
N29	heptadecanenitrile	251	57+97	2.76	0.12	0.00	0.00	0.00
N30	Eicosanenitrile	293	57+97	3.03	0.04	0.00	0.00	0.00
PA1	1H-indene. 3-methyl	130	115+130	1.118	0.31	0.00	0.06	0.02
PA2	naphtalene, 1,2-dihydro-2-methyl	144	129+144	1.354	0.15	0.01	0.03	0.01
PA3	naphtalene, 1,6-dimethyl	156	141+156	1.675	0.21	0.01	0.05	0.02
PA4	tetramethyl phenanthrene	234	219+234	2.941	0.33	0.00	0.04	0.00
Ph1	resorcinol	110	54+110	0.752	1.44	0.22	0.5	0.26
Ph2	phenol	94	66+94	0.782	7.61	1.10	8.59	2.73
Ph3	2-methyl phenol	108	107+108	0.932	1.99	0.05	0.41	0.14
Ph4	3/4-methyl phenol	108	107+108	0.982	24.08	0.03	2.8	1.97
Ph5	methoxy-1,2-benzenediol	140	125+140	1.128	1.29	0.03	0.00	0.00
Ph6	phenol, 2-6-dimethyl	122	107+122	1.13	0.84	0.06	0.29	0.08
Ph7	4-hydroxy acetophenone	136	93+121	1.156	0.46	0.07	0.07	0.04
Ph8	phenol, 3-ethyl	122	107+122	1.181	2.24	0.10	0.75	0.45
Ph9	3-methoxy-1,2-benzenediol	140	125+140	1.393	2.7	0.00	1.72	0.94
Ph10	phenol methylenebis	200	199+200	2.358	0.17	0.01	0.04	0.03
Ps1	2-methylfuran	82	53+82	0.223	5.46	0.22	1.19	0.91
Ps2	acetic acid	60	45+60	0.273	19.48	0.90	11.83	6.95
Ps3	2,5-dimethylfuran	96	95+96	0.291	2.76	0.03	0.27	0.15
Ps4	(2H)-furan-2-one	84	54+84	0.404	5.67	0.10	1.05	0.47
Ps5	2-ethyl-5-methylfuran	110	95+110	0.409	9.05	0.00	0.12	0.03
Ps6	3-furaldehyde	96	95+96	0.451	39.05	0.07	5.85	4.26
Ps7	3-methylfuran/2-cyclopenten-1-one	96	53+82	0.471	3.01	0.02	9.00	0.69
Ps8	2-cyclopentenone 2methyl	96	67+96	0.598	1.05	0.02	0.38	0.31
Ps9	2-acetylfuran	110	95+110	0.605	3.77	0.09	1.02	0.41
Ps10	dihydro-3-methylene-2(3H)-furanone	98	98+68	0.647	1.14	0.00	0.00	0.00
Ps11	2,3-dihydro-5-methylfuran-2-one	98	55+98	0.671	0.82	0.00	6.08	2.82

Appendix A. (continuation)

Code	Name	M	m/z	RT-GUAIACOL (min)	% TICsoils (max)	% TICsoils (min)	% TICveg (max)	% TICveg (min)
Ps12	5-methyl-2-furaldehyde	110	109+110	0.714	16.72	0.05	1.35	0.80
Ps13	2-cyclopenten-1-one, 3-methyl	96	96+67	0.726	0.88	0.00	0.54	0.33
Ps14	3-hydroxy-2-methyl-2-cyclopentene	112	55+112	0.877	1.78	0.12	2.70	2.09
Ps15	dianhydro rhamnose	128	113+128	0.882	1.86	0.01	0.11	0.03
Ps16	2,3-dimethylcyclopent-2-en-1-one	110	67+110	0.885	0.62	0.00	0.27	0.19
Ps17	benzofuran, 2-methyl-	132	131+132	1.028	0.61	0.06	0.07	0.03
Ps18	levoglucosenone	126	68+98	1.054	5.34	0.00	0.11	0.02
Ps19	maltol	126	71+126	1.068	3.22	0.30	1.04	0.72
Ps20	dimethyl benzofuran	146	145+146	1.249	0.46	0.05	0.07	0.02
Ps21	1,4:3,6-dianhydro-alpha-D-glucopyranose	144	57+69	1.265	1.99	0.00	0.00	0.00
Ps22	methyl 2,4-di-o-methyl-β-D-xylopyranoside?	192	74+101	1.697	2.42	0.07	0.00	0.00
Ps23	levomannosan	162	60+73	1.825	1.59	0.00	0.62	0.28
Ps24	levoglucosan	170	60+73	1.993	11.57	0.09	2.54	0.86
Ps25	C14alkylfuran	264	81+95	2.482	0.39	0.03	0.00	0.00
St1	pregn-4-ene-3,20-dione compound	346	121+299	3.333	0.34	0.00	0.00	0.00
St2	sterol	n.d.	161+351	3.733	0.46	0.00	0.01	0.00
St3	neooleana compound	n.d.	365+175	3.745	0.10	0.00	0.00	0.00
St4	beta-amyrin	426	203+218	3.849	0.17	0.00	0.01	0.00
St5	gamma -tocopherol	416	151+416	3.851	0.07	0.00	0.06	0.01
St6	28-norolean-17-en-3-one compound	410	163+190	3.864	0.40	0.00	0.13	0.00
St7	stigmastan-3-ol compound	492	147+396	3.898	0.16	0.00	0.16	0.06
St8	sterol	n.d.	121+393	4.130	0.70	0.00	0.01	0.00
St9	sterol	394	393+394	4.133	3.46	0.00	0.00	0.00
St10	stigmasta-3, 5-dien-7-one	410	174+410	4.203	0.20	0.00	0.03	0.01
Tp1	squalene	410	69+81	3.607	0.09	0.00	0.12	0.01
Tp2	dehydroabietic acid. methyl ester 1	314	239+240	2.629	0.08	0.00	0.04	0.00
Tp3	dehydroabietic acid. methyl ester 2	314	239+240	3.084	0.08	0.00	0.18	0.00

M = molecular weight, *m/z* = mass ions used for quantification, RT = relative retention time (to guaiacol)

CHAPTER 3

Molecular characteristics of the soil organic matter fraction resistant to permanganate- and dichromate- oxidation

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List of authors:

MANUEL SUÁREZ-ABELENDA[a], JOERI KAAL[b], HEIKE KNICKER[c], MARTA CAMPS-ARBESTAIN[d], FELIPE MACÍAS[a]

[a]Departamento de Edafoloxía e Química Agrícola, Facultade de Bioloxía, Universidade de Santiago de Compostela, 15782- Santiago de Compostela, Spain

[b]Instituto de Ciencias del Patrimonio (Incipit), Consejo Superior de Investigaciones Científicas (CSIC), Rúa San Roque 2, 15704 Santiago de Compostela, Spain.

[c]Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Adva. Reina Mercedes, 10, 41012 Sevilla, Spain.

[d]Institute of Natural Resources, Private Bag 11222, Massey University, Palmerston North 4442, New Zealand.

Molecular characteristics of the soil organic matter fractions resistant to permanganate- and dichromate- oxidation

Summary

Samples from a black C- rich colluvial soil in NW Spain were subjected to $K_2Cr_2O_7$ and $KMnO_4$ oxidation and the residual soil organic matter (SOM) was NaOH-extracted and analyzed using analytical pyrolysis-gas chromatography-mass spectroscopy (Py-GC-MS) and solid-state ^{13}C cross polarization-magic angle spinning-nuclear magnetic resonance (^{13}C CP MAS-NMR) in order to study the susceptibility of different SOM fractions (fresh, microbial, pyrogenic and root-derived) towards these oxidation agents. Non-oxidized samples following the same NaOH-extraction procedure were also analyzed. Py-GC-MS and ^{13}C NMR indicated that $KMnO_4$ promotes the oxidation of carbohydrate products, mostly from (i) microbial SOM and (ii) a resistant lignocellulose fraction, causing a relative enrichment of aliphatic moieties and aromatic black C structures. From Py-GC-MS, residual SOM after $K_2Cr_2O_7$ oxidation contained black C, N-containing black C markers and aliphatic structures resistant against this oxidant. This was corroborated by a relatively intense resonance of aromatic C and some signal of alkyl C in ^{13}C NMR spectra. These results confirm that dichromate oxidation residues contain a non-pyrogenic fraction mainly consisting of long-chain aliphatic structures.

1. Introduction

Soil organic matter (SOM) is a complex mixture of plant, animal and microbial tissue, both fresh and at different stages of decomposition (Stevenson, 1994; Tabatabai, 1996). The molecular structures present in SOM have been a source of debate for many decades due the analytical difficulties inherent to SOM characterization (Piccolo, 1996). A wide set of methodologies have been used to study SOM composition, including (i) chemolytic techniques (i.e. application of acid-hydrolysis or extracting agents) coupled with colorimetric and/or GC-MS analyses to identify specific SOM components (polysaccharides, lignin-derived compounds, amino sugars, extractable- lipids or hydrolysable proteins) (Kögel-Knabner, 1995); (ii) physical fractionation into organo- mineral fractions based on particle size and/or density yields (Christensen, 1992; Six et al., 2002); (iii) wet oxidation with potassium permanganate ($KMnO_4$) (Loginow et al., 1987; Blair et al., 1995; Tirol-Padre and Ladha, 2004), H_2O_2 (Eusterhues et al., 2005), $Na_2S_2O_8$ (Eusterhues et al., 2003), NaOCl (Kleber et al., 2005), and $K_2Cr_20_7$ (Skjemstad and Taylor, 1999); and (iv) spectroscopic techniques, such as infrared spectroscopy, Py-GC-MS and ^{13}C CP MAS-NMR (Wilson et al., 1981; Fründ et al., 1993; Knicker, 2011). The latter two are probably the most useful tools to obtain detailed information of complex mixtures of organic compounds such as SOM. Py-GC-MS is based on thermal degradation in an inert atmosphere (pyrolysis) and subsequent separation (GC) and identification (MS) of the pyrolyzate, from which information on macromolecular structures can be extracted (Moldoveanu, 1998). For example, it allows the identification of sources of plant detritus, microbial material, pyrogenic material (black C; BC), estimation of contributions of these components and recognition the degradation state of SOM and individual SOM components (Nierop et al., 2005; Buurman et al., 2007; Buurman and Roscoe, 2011). Solid-state ^{13}C NMR analysis with cross polarization-magic angle spinning (CP-MAS) provides information on the distribution of functional groups containing C giving signals in specific chemical shift regions (Wilson, 1987; Baldock and Smernik, 2002), which can be quantified by integration. Although often supposed to underestimate BC, recent studies demonstrated that most charcoals have an atomic H/C ratio >0.5 and thus provide sufficient protonation for efficient cross polarization and reliable NMR spectra (Knicker et al., 2005a).

The Walkley-Black dichromate extraction, as modified by Heanes (1984), is a relatively simple and rapid procedure with minimal equipment needs (Nelson and Sommers, 1996) that has long been used to estimate the organic C (OC) content of soils. Its major disadvantage is that it incompletely oxidizes soil OC (Gillman et al., 1986; Lowther et al., 1990), and has different oxidation efficiencies among in different soils (Tabatabai, 1996), which produce considerable and unpredictable deviations from 'true' soil OC content. Probable causes for these low recoveries are (i) spatial inaccessibility of organic substrates to the oxidation agent (Skjemstad et al., 1996; Six et al., 2002; von Lützow et al., 2006; Buurman and Roscoe, 2011), (ii) binding with inorganic phases (Sollins et al., 1996; Eusterhues et al., 2005) and (iii) the presence of chemically recalcitrant SOM fractions such as charred material (Goldberg, 1985; Six et al., 2002; von Lützow et al., 2006). In fact, the difference between total OC and dichromate-oxidizable OC has been used to estimate the BC content of soils. This is defined as the product resulting from incomplete thermal combustion of vegetation and/or fossil fuels that is relatively resistant to decomposition (Schmidt et al., 2001). However, an unknown portion of BC is actually oxidized while some non-pyrogenic SOM may survive the oxidation, e.g. non-hydrolysable aliphatic compounds that resist aqueous dichromate oxidation (Knicker et al., 2007). This implies that the properties of the oxidation-resistant residue must be assessed in order to obtain meaningful estimations of BC content using dichromate oxidation (Knicker et al., 2007).

The permanganate-oxidizable fraction has been used as a proxy of the labile fraction of SOM (Loginow et al., 1987, Lefroy et al., 1993; Blair et al., 1995), based on the assumption that the oxidative capacity of $KMnO_4$ on SOM is comparable to that of soil microbial enzymes (Conteh et al., 1997). However, some studies indicated that $KMnO_4$- oxidizable C may not be a reliable measure of the proportion of labile C because, even though it efficiently degrades lignin and glycol-based aromatic compounds, it has little effect on several SOM components that are widely recognised as easily degraded by soil microorganisms, e.g. structural carbohydrates, sugars and amino acids (e.g. Tyrol-Padre and Ladha, 2004).

SOM is thermodynamically unstable in well-aerated soils (Macías and Camps-Arbestain, 2010). However, SOM stabilized by specific mechanisms can remain as meta-stable forms in soils for hundreds to thousands of years (Six et al., 2002). These non-ideal conditions to SOM decay are associated to physical and chemical protection mechanisms offered by the soil matrix that either impede the access of enzymes to SOM (e.g., within microaggregates or by creating hydrophobicity) or increase the energy needed to degrade SOM through interactions with minerals (Eusterhues et al., 2003; von Lützow et al., 2006). In addition, enhanced SOM preservation may occur when environmental conditions are not adequate for microbial growth, e.g. the presence of free Al and Fe, low soil pH and/or due to low nutrient availability (Buurman and Roscoe, 2011). Furthermore, intrinsic recalcitrance of specific SOM components (i.e. by presence of high bond strength) may increase its longevity in soil. This is most likely the main process responsible for the long turn-over of highly condensed aromatic compounds present in BC (Harvey et al., 2012).

This study aims to define the molecular characteristics of $KMnO_4$- and $K_2Cr_2O_7$-oxidation-resistant SOM by Py-GC-MS and ^{13}C CP MAS-NMR, which may help to understand the stability of specific organic compounds against soil microbial oxidative enzymes. Samples were collected from a BC-rich haplic umbrisol, representing ages of ca. 100, 5000 and 9700 years under different vegetation, allowing us to focus on the behavior of BC towards these oxidation agents.

2. Material and methods

2.1. Study site and sample descriptions

Soil PRD-4 is a 2.4 m thick haplic umbrisol (humic/alumic) according to the IUSS Working Group (2006) and humic pachic dystrudept according to the SSS (1998). This soil type is traditionally referred to as Atlantic Ranker (Carballas et al., 1967). Radiocarbon dating showed that the soil gradually accumulated through colluviation during the last ca. 13 ky (Kaal et al., 2011). Soil PRD-4 has a deep black color owing to a combination of high SOM content and abundance of charred residues (Table 1). A depiction of the soil profile and its

physicochemical characterization can be found in Kaal et al. (2008a). For the present study, three samples were selected from this soil, corresponding to three periods with radically different ecosystems and hypothetically also SOM compositions: S1 (5-10 cm depth) corresponds to recent material (<150 y BP), evidenced by ^{14}C dating and the presence of pollen of exotic species *Eucalyptus sp.* (López-Merino et al., 2012) (Table 1). This soil layer contains considerable amounts of "fresh" (non- or slightly degraded) SOM, as described by Kaal and van Mourik (2008) and root fragments. The vegetation corresponding to this period is a mosaic of shrubland (dominated by Ericaceae), pasture and exotic tree species. Sample S2 (95-100 cm depth) contains large amounts of charcoal from palaeofires (Kaal et al., 2011) that occurred ca. 5 ky ago. Anthracological analysis showed that most charcoal originates from deciduous *Quercus sp.* This sample is thought to correspond to an oak-dominated woodland under substantial fire and grazing pressure. Sample S3 (190-195 cm depth) corresponds to an Early-Holocene phase (ca. 9.7 ky ago) with "steppe-like" vegetation dominated by *Betula sp.* (birch), shrubs of the Fabaceae family and herbaceous species that preceded the colonization of the area by deciduous forest.

2.2. Determination of organic C fractions

Potassium dichromate-oxidizable organic C (OC_{dichro}) was determined following the Walkley-Black oxidation method as modified by Wolbach and Anders (1989) and Knicker et al. (2007). 0.5 g of dry soil (< 2 mm) were oxidized in triplicate with 20 ml of 0.2 M $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4, in addition to control samples that contained no soil, at 60 °C in a water bath for 6 h. After the reaction, excess dichromate was determined by titration against 0.033 M $FeSO_4$. The amount of dichromate consumed by the soil was used to calculate the amount of dichromate-oxidizable organic C (OC_{dichro}) assuming that (i) the oxidation state of soil OC is zero (C^0) and (ii) complete oxidation to C^{+4} occurs, even though the oxidation states of soil OC have been reported to range from –0.45 to 0.3 (Kleber, 2010).

Potassium permanganate-oxidizable organic C (OC_{per}) was determined, in triplicate, using 25 ml of 33 mM $KMnO_4$ solution added in 50 ml centrifuge tubes containing an amount of dry soil (<2 mm) equivalent to 15 mg organic C (Tirol-Padre and Ladha, 2004). After 24 h

shaking, the tubes were centrifuged for 5 min at 2600 *g* and the supernatant diluted in distilled water (2:50 v:v). Absorbance was read on a split beam spectrophotometer at 565 nm. Blanks and a standard soil were analyzed before each run. For calculation purposes, it was assumed that three moles of C are oxidized for every four moles of Mn^{+7} reduced (Tirol-Padre and Ladha, 2004).

Table 1. General information of samples studied from soil PRD-4. OC_{per} is permanganate-oxidizable organic C, OC_{dichro} is dichromate/oxidizable

Sample	S1	S2	S3
depth	5-10 cm	95-100 cm	190-195 cm
conventional ^{14}C age BP	104.3 ± 0.4 pMC (present)	4090 ± 30	9760 ± 50
radiocarbon sample code	Ua-34719	β-299230	β-240963
C [mg g^{-1} soil]	62.3	36.7	42.9
OC_{per} [mg g^{-1} soil]	2.3	1.3	1.7
OC_{dichro} [mg g^{-1} soil]	46.7	29.3	34.3
C/N (atomic) [-]	15.9	24.4	23.9
pH-H_2O [-]	4.6	5.0	5.2
charcoal >2 mm [mg g^{-1} soil]	0.03	1.97	0.07

2.3. Isolation of SOM fractions

The non-oxidized (NO) SOM extraction was considered the control treatment and was extracted following the methodology for SOM extraction and purification prior to Py-GC-MS as described by Buurman et al. (2007). For this, 5 g soil (air-dried fine earth <2 mm) was extracted with 50 ml of 1 M NaOH and shaken for 24 h under N_2 to prevent oxidation/saponification. The suspension was centrifuged at 2600 *g* for 1 h and the extract decanted, after which the extraction was repeated. The two extracts were combined and the residues discarded. The extracts were then acidified to pH 1 with concentrated HCl to protonate SOM. One ml of concentrated HF was added to dissolve silicates and increase C content of the extracted fraction. The acid mixture was shaken for 48 h, after which it was dialyzed to neutral pH against distilled water to remove excess salt. Finally, the suspension was freeze-dried.

Dichromate oxidation-resistant SOM (CR): 12.5 g soil was oxidized in 500 ml of 0.2 M $K_2Cr_2O_7$ and 100 ml of concentrated H_2SO_4 for 6 h at 60 °C using a water bath. Once cooled,

the suspension was centrifuged at 2600 *g* for 1 h and the supernatant decanted, after which the sediment was washed with distilled water until the solution was colorless. The suspension was discarded and the dichromate oxidation-resistant SOM extracted by 200 ml of 1 M NaOH for 24 h under N_2 flow. The resultant suspension was centrifuged at 2600 g for 1 h and the supernatant decanted. This extraction was repeated twice. Thereafter, the three extracts were combined and acidified to pH 1 with concentrated HCl. One ml of concentrated HF was added to dissolve silicates and increase the content of organic C of the extracted fraction. This acid mixture was shaken for 48 h, dialyzed against H_2O to neutral pH and finally freeze- dried.

Permanganate oxidation-resistant SOM (MN): 1000 ml of 33 mM $KMnO_4$ were added to 4.8, 8.2 and 7.0 g dry soil (<2 mm) for samples S1, S2 and S3, respectively, aiming to add 25 ml of $KMnO_4$ per 15 mg of organic C (calculated from OC_{dichro} values). After 24 h shaking, the suspension was centrifuged at 2600 *g* for 1.5 h and the extract decanted, after which the sediment was washed with distilled water until the supernatant was colorless. The MN in the residue was isolated and purified using 200 ml of 1 M NaOH for 24 h under N_2 atmosphere, analogous to the extraction of CR described above.

2.4. Py-GC-MS

Platinum filament Py-GC-MS was performed with a Pyroprobe 5000 (CDS Analytical Inc., Oxford, USA) coupled to a 6890 GC and 5975 MS (Agilent Technologies, Palo Alto, USA). The non-oxidized (NO) and oxidation-resistant SOM fractions (MN and CR) were pyrolyzed at 750 °C for 10 s (heating rate 10 °C/ms). Analyses of sample S2 of the CR series was repeated, first at 400 ºC and then at 750 ºC, to distinguish between evaporation and pyrolysis products from volatile and macromolecular components, respectively. The pyrolysis interface was set at 300 ºC and the GC inlet at 325 °C. The oven of the GC was heated from 50 to 325 °C at 10 °C/min and held isothermal for 5 min. The GC-MS transfer line was held at 325 °C, the ion source (in electron impact mode, 70 eV) at 230 °C and the quadrupole detector at 150 °C, measuring fragments in the *m/z* 50-500 range. The GC was equipped with a (non-polar) HP-1 100% dimethylpolysiloxane column. Helium was used as the carrier gas (constant gas flow, 1 ml/min). The major peaks in the total ion current of all samples were listed and, if

possible, identified using the NIST '05 library and Py-GC-MS literature (Appendix A). Quantification of these pyrolysis products, 172 in total, was obtained by using the peak area of the major fragment ions (m/z). The sum of these peaks, i.e. total quantified peak area (TQPA) was set as 100% and the relative proportions of the pyrolysis products were calculated as the % of TQPA. This is a semi-quantitative estimate that allows for better comparison among samples than visual inspection of pyrolysis chromatograms (pyrograms) alone, and produces a dataset that can be treated statistically.

2.5. *Solid-state ^{13}C NMR spectroscopy*

Samples were analyzed by solid- state ^{13}C NMR spectroscopy using a Bruker AMX300 spectrometer operating at a frequency of 75 MHz. The cross polarization magic-angle spinning (CP-MAS) technique was applied with a spinning speed of 14 kHz. A ramped ^{1}H-pulse was used during the contact time of 1 ms to avoid spin modulation during the Hartmann-Hahn contact. According to former publications, demonstrating short ^{1}H-spin-lattice relaxation times for humified SOM (Knicker, 2011), pulse delays of 300 ms were used for all spectra. Because of low C content 200,000 scans were accumulated. Line broadenings of 100 Hz were applied. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm) using glycine (COOH: 176.08 ppm). Spectra were divided into different regions of chemical shift following Knicker et al. (2005b). Relative abundances of the various C groups were determined by integration of the signal intensity in their respective chemical shift regions. The region between 0-45 ppm is assigned to alkyl C corresponding to terminal methyl groups and methylene groups of aliphatic moieties. The *O*-alkyl C region, typically assigned to carbohydrate-derived structures, between 45-95 ppm. Here, between 45-60 ppm, *N*-alkyl C (i.e. in amino sugars and peptide structures) can contribute to the signal. Between 90 and 160 ppm resonance lines of olefins and aromatic C are detected. The regions from 160 to 220 ppm and from 220 to 245 are assigned to carbonyl C separated into carboxyl/amide and aldehyde/ketone groups, respectively. Because of the limited sample availability some samples required Al-oxide to fill the rotor, causing some signal quality deterioration. Samples MN-2, MN-3 and CR-3 were not analyzed for that reason.

2.6. Factor analysis

The relative proportions of pyrolysis products were subjected to factor analysis using Statistica Version 8 (Statsoft, Tulsa, USA). Factor analysis proved useful in the interpretation of Py-GC-MS datasets, especially with respect to the sources and degradation states to which the pyrolysis products correspond (Windig et al., 1980).

3. Results and discussion

3.1. Py-GC-MS: source allocation

The pyrolysis products were grouped according to their chemical structure into the following classes: (i) aliphatic compounds (homologous series of *n*-alkanes and *n*-alkenes, and branched alkenes), (ii) lignin-derived methoxyphenols, (iii) phenols, (iv) monocyclic aromatic compounds (MAHs), (v) polycyclic aromatic hydrocarbons (PAHs), (vi) N-containing compounds, (vii) carbohydrate-derived pyrolysis products and (viii) unidentified compounds. Appendix A is a list of all pyrolysis products identified.

3.1.1. Aliphatic compounds. *n*-Alkane/*n*-alkene pairs ranging from C_{10} to C_{28} originate largely from aliphatic biopolymers (Eglinton and Hamilton, 1967; Killops and Killops, 2005). In addition, branched alkenes and alkenes with unknown molecular conformation, with a probable C number in the range 10-20, were detected. Recent studies suggest that these short- and mid-chain branched aliphatic pyrolysis products originate from charred aliphatic precursors (Eckmeier and Wiesenberg, 2009; Wiesenberg et al., 2009; Calvelo-Pereira et al., 2011; Kaal et al., 2012a). Several *n*-fatty acids (mainly C_{16} and C_{18}) seemed to increase disproportionally upon chemical oxidation, especially in CR. This might be explained by enrichment of aliphatic structures upon dichromate oxidation due to their hydrophobicity (Knicker et al., 2007) but the use of a HP-1 column, which has a larger internal diameter than frequently used non-polar columns for Py-GC-MS, may have affected the 'chromatographic mobility' and relative proportions of these compounds, which is why they cannot be reliably interpreted in this case. These compounds were not included in the statistical analyses.

3.1.2. Lignin-derived methoxyphenols. Methoxyphenols (guaiacyl- and syringyl-based) are typical products of coniferyl and sinapyl lignin, respectively (Boerjan et al., 2003). 4-vinylphenol was also added to this group because it has frequently been shown to be marker of coumaryl lignin in grasses (del Río et al., 1996; Sáiz-Jiménez and de Leeuw, 1986). An unknown proportion of the 4-vinylphenol and 4-vinylguaiacol may originate from non-lignin phenolic acids (Schellekens et al., 2012), but that does not influence the interpretation of results here as statistically they behave as lignin markers.

3.1.3. Phenols. These other phenols have multiple origins: phenol and C_1-C_2-alkylphenols originate from any phenolic precursor including lignin, tannin, proteinaceous biomass, weakly-charred BC and carbohydrates (Tegelaar et al., 1995; Stuczynski et al, 1997; van Heemst et al., 1999), while lignin, tannin or thermally demethylated lignin (Kaal et al., 2012b) are the most likely precursors of 1,2-benzenediol (catechol).

3.1.4. Monocyclic aromatic hydrocarbons. MAHs include benzene, toluene, styrene, dimethylbenzenes, linear C_2-C_4-alkylbenzenes, a dimethylstyrene and a dimethyl-methylethylbenzene compound. MAHs are formed from many aromatic and some non-aromatic precursors but BC is known to produce an exceptionally high proportion of especially benzene (Kaal et al., 2012a). The alkylstyrenes most likely originate from the monoterpenes present in *Eucalyptus globulus* litter (see below).

3.1.5. Polycyclic aromatic hydrocarbon compounds. The origin of PAHs in SOM pyrolyzates have been the subject of considerable debate. They were once considered as evidence of condensation reactions during humification (Schulten et al., 1991), then interpreted as an analytical artefact because of evidence of their formation during pyrolysis of aliphatic compounds including *n*-fatty acids through cyclization reactions (Sáiz-Jiménez, 1994; Almendros, 2008) and, more recently, PAHs and particularly the non-alkyl-substituted and >2 ring PAHs (Rumpel et al., 2007), are considered indicative of (but not markers of) BC in SOM (Kaal and Rumpel, 2009; Song and Peng, 2010). In the present study, unsubstituted PAHs (indene, naphthalene, fluorene, biphenyl, phenanthrene and anthracene) and C_1-C_2 alkyl analogues of these PAHs were abundant. In addition, a series of C_3-C_4 alkylnaphthalenes and

$C_{5:0}$ and $C_{5:1}$ alkylnaphthalenes probably originate from evaporation and pyrolysis of monoterpenes (e.g. pinene, phellandrene, eucalyptol) and sesquiterpenoids (aromadendrene, globulol), respectively, present in *Eucalyptus sp.* oil. Indeed, these compounds were identified in *Eucalyptus globulus* litter (leaf, cortex and branches) pyrolyzates (data not shown). Some of the polysubstituted PAHs (C_3-indene, C_2-$C_{5:0}$- and $C_{5:1}$-naphthalene) provided the largest contributions to the pyrograms of S1 (contrary to many unsubstituted or C_1- alkylsubstituted PAHs from BC); this suggests that a significant portion of these compounds originate from fresh *Eucalyptus sp.* litter.

3.1.6. N-containing products. Of the 24 N-containing pyrolysis products identified, benzonitrile and C_1-benzonitriles were recently proposed as the main products of N-containing groups in BC (Schnitzer et al., 2007; Song and Peng, 2010). In addition, isoquinoline, phenylpyridine, benzenedicarbonitriles and pyridinecarbonitriles can be considered as markers of 'black N' (BN) (Knicker, 2007; Kaal et al., 2009). Note that absence of these products does not imply absence of BN: these compounds can probably only be detected in high- quality programs of BN-rich samples. Pyrroles, pyridines and indoles are potential products of BN as well but these compounds are common in the pyrolyzates of non-pyrogenic N-moieties. Several markers of chitin (acetamide and a compound tentatively identified as trianhydro-2-acetamido-2-deoxyglucose; van der Kaaden et al., 1984; Stankiewicz et al., 1996) and chitin-entangled protein (diketopiperazine) probably originate from fungal cell walls and/or arthropod exoskeleta, either way serving as an indication of biologically re-assimilated remains in SOM (Gutierrez et al., 1995). Finally, for picolinamide, cyanobenzoic acid and the phthalimide-based compounds no specific origin has been identified yet.

3.1.7. Carbohydrate compounds. Of the carbohydrate products identified, levoglucosan, dianhydro-α-glucopyranose, pyranones and dianhydrorhamnose largely originate from "fresh" or well- preserved polysaccharides (Stuczynski et al, 1997; Poirier et al., 2005; Nierop et al., 2005). On the other hand, cyclopentenediones, furans, furfurals, levoglucosenone and dibenzofuran originate from fresh and/or degraded carbohydrates (Buurman and Roscoe, 2011).

This degradation may be either biological or thermal in nature, the latter especially for the furans, furaldehydes and dibenzofuran (Pastorova et al., 1994; Boon et al., 1994).

3.1.8. Unidentified compounds. An unsaturated non-aromatic cyclic compound (U1) was identified only in the pyrolyzate of sample S1, which also contained the pollen of *Eucalyptus sp.* It probably corresponds to α-phellandrene, which is abundant in eucalyptus oils (Samate et al., 1998). Furthermore, several polymethyl-substituted polycyclic compounds (U3-U6), also detected in the aforementioned fresh eucalyptus-litter pyrolyzate, probably derived from *Eucalyptus sp.* Finally, a methylated cyclohexane (U2) of unknown origin was tentatively identified.

3.2. Py-GC-MS: quantification results and interpretation

The relative contributions to TQPA for identified groups in the different soil horizons studied are presented in Table 2. In NO-1, carbohydrate-derived pyrolysis products accounted for 30% of TQPA, with levoglucosan (Ps13) from intact polysaccharide (Stuczynski et al, 1997; Poirier et al., 2005) being dominant. The presence of 4-hydroxy-5,6-dihydro-(2H)-pyranone (Ps6) and dianhydrorhamnose (Ps7) confirms the existence of fresh (or well-preserved) polysaccharides in NO-1 (Nierop et al., 2005). Of the samples studied, these compounds showed the largest contribution to the NO-1 sample. The same pattern was observed for many other indicators of fresh plant material, including the aliphatic compound producing *m*/*z* 83+280, diketodipyrrole, and the lignin-derived products (Buurman et al., 2009; Suárez-Abelenda et al., 2011). This compendium suggests that the abundant phenols detected in this sample originate from lignin as well. Samples NO-1 and MN-1 had the highest contributions of eucalyptus-derived moieties ($C_{5:0}$-, $C_{5:1}$-alkylnaphthalenes and α-phellandrene) and C_3- naphthalenes. It is concluded that the SOM of NO-1 is characterized by a large fraction of well- preserved polysaccharides and lignin, with an additional major contribution of specific eucalyptus-derived substances, and low values of microbial and pyrogenic SOM. The latter is supported by the low benzene/alkyl-benzenes and PAH/alkyl-PAHs ratios (Table 2), which are indicative of a low contribution of strongly-charred BC to the MAHs and PAHs of these samples (Kaal and Rumpel, 2009; Kaal et al., 2012a).

Unsurprisingly, the ca. 5 ky old NO-2 sample produced fewer pyrolysis products from fresh SOM than the NO-1 sample. More specifically, in comparison with NO-1, among the carbohydrate markers there was a strong increase of furans, furaldehydes, levoglucosenone and acetic acid, while levoglucosan, pyranones and dianhydrorhamnose diminished, which is a clear indication of a shift of plant- to microbial-derived carbohydrates (Sáiz-Jiménez and de Leeuw, 1986; Buurman et al., 2007; Buurman and Roscoe, 2011). Lignin markers were virtually absent. NO-2 sample produced many more N-compounds, including those from chitin (N3 and N22), pyridine (N1, often associated with microbial SOM; Buurman et al., 2007) and BN (e.g. aromatic carbonitriles and phenylpyridine). It also gave higher proportions of MAHs and BC-derived PAHs than the NO-1 sample. It is concluded that the SOM of sample NO-2 was predominantly composed of microbial/fungal SOM and BC.

The pyrolyzate of sample NO-3 was dominated by carbohydrate markers, with acetic acid, 3/2-furaldehyde, 5-methyl-2-furaldehyde, dianhydro-α-glucopyranose, a furanone and 4-acetylfuran accounting for 62% of TQPA (Table 2). These pyrolysis products are frequently ascribed to SOM with high loads of microbial biomass (Sáiz-Jiménez and de Leeuw, 1986; Buurman et al., 2007; Buurman and Roscoe, 2011). The small relative proportions of MAHs and PAHs suggests that BC accounts for only a minor portion of the SOM in NO-3. This is supported by the low ratios of benzene/alkyl-benzenes and PAH/alkyl-PAH (Table 2).

In general, the differences in pyrolyzate compositions between NO-samples and MN-samples were small, yet some are worth mentioning. For sample S1, oxidation with $KMnO_4$ caused an increase in MAHs (from 16.4% in NO-1 to 30.1% in MN-1) and decrease in carbohydrates (from 30.4% to 18.4%) and lignin (from 7.4% to 3.0 %) (Table 2). These results can be explained by the partial oxidation of fresh SOM (van Soest and Wine, 1986; Tirol-Padre and Ladha, 2004) and the relative enrichment of pyrogenic and aliphatic SOM. In the ca. 5 ky old sample, $KMnO_4$ oxidation (MN-2) caused a strong decline in carbohydrate products (from 31.5% to 17.4% of TQPA in NO-2 and MN-2, respectively) and an increase in aliphatic pyrolysis products (sum of *n*-alkanes,

Table 2. Relative contributions of pyrolysis product groups and benzene/alkylbenzenes and PAH/alkyl-PAHs ratios of total quantified peak area (% TQPA)

		total n-enes	n-enes >C_{18}	n-enes C_{18}-C_{10}	total n-anes	n-anes >C_{18}	n-anes C_{18}-C_{10}	other aliph^{+}	phs	C_2-ph	total Lg	total MAHs	alkyl-B	total PAHs	ITPB-PAHs	BC-PAHs	total N	BN	total Ps	well pres Ps	degr Ps	U	benz/alkyl-benz	PAH/alkyl-PAHs
NO-1	TQPA %	**1.7**	0.9	0.8	**1.9**	0.8	1.1	1.4	**16.6**	1.8	**7.4**	**16.4**	6.6	**10.6**	10.1	0.5	**10.7**	3.6	**30.4**	17.8	12.4	2.5	0.2	0.02
	%*		*51.4*	*48.6*		*40.1*	*59.9*						*40.0*		*95.6*	*4.4*		*33.2*		*58.4*	*40.7*			
MN-1	TQPA %	**1.6**	0.5	1.1	**2.0**	0.3	1.7	4.1	**14.1**	1.9	**3.0**	**30.0**	13.4	**12.3**	11.4	1.0	**11.7**	4.8	**18.4**	10.4	7.7	2.7	0.2	0.04
	%*		*33.6*	*66.4*		*13.4*	*86.6*						*44.7*		*92.2*	*7.8*		*40.7*		*56.6*	*41.7*			
CR-1	TQPA %	**9.3**	3.0	6.3	**10.2**	3.3	6.9	14.6	**10.9**	1.4	**1.0**	**26.3**	4.6	**4.8**	2.2	2.6	**15.8**	7.4	**6.4**	0.8	5.4	0.7	1.2	0.60
	%*		*32.2*	*67.8*		*32.2*	*67.8*						*17.5*		*45.7*	*54.3*		*47.0*		*12.4*	*84.7*			
NO-2	TQPA %	**1.8**	0.6	1.2	**1.8**	0.6	1.2	1.7	**13.7**	0.8	**1.8**	**20.4**	4.9	**5.7**	4.2	1.5	**20.9**	11.9	**31.5**	4.0	27.1	0.7	0.9	0.21
	%*		*34.2*	*65.8*		*34.1*	*65.9*						*23.8*		*73.0*	*27.0*		*57.1*		*12.6*	*86.3*			
MN-2	TQPA %	**2.5**	0.7	1.9	**3.2**	0.5	2.7	17.4	**11.6**	0.9	**1.4**	**20.4**	3.6	**3.3**	2.2	1.2	**22.2**	13.9	**17.4**	3.3	13.6	0.5	1.3	0.26
	%*		*26.0*	*74.0*		*16.7*	*83.3*						*17.5*		*65.0*	*35.0*		*62.6*		*18.7*	*77.9*			
CR-2	TQPA %	**4.6**	1.6	3.0	**2.5**	0.9	1.5	7.0	**4.8**	0.5	**0.8**	**16.9**	2.0	**3.4**	0.9	2.5	**32.2**	23.8	**27.6**	15.7	11.5	0.2	3.9	1.31
	%*		*35.3*	*64.7*		*37.7*	*62.3*						*11.6*		*25.3*	*74.7*		*74.1*		*57.0*	*41.8*			
NO-3	TQPA %	**0.9**	0.3	0.5	**1.0**	0.5	0.5	1.5	**6.6**	0.0	**0.7**	**10.3**	2.5	**1.4**	0.9	0.5	**15.0**	9.0	**62.2**	2.7	59.2	0.3	1.3	0.35
	%*		*36.6*	*63.4*		*47.3*	*52.7*						*24.0*		*65.0*	*35.0*		*60.2*		*4.4*	*95.2*			
MN-3	TQPA %	**2.7**	0.8	1.9	**2.9**	1.2	1.7	9.2	**7.7**	0.0	**1.2**	**17.5**	4.1	**2.4**	1.4	1.0	**38.3**	28.9	**17.7**	1.1	16.0	0.4	0.8	0.34
	%*		*30.2*	*69.8*		*42.0*	*58.0*						*23.2*		*57.9*	*42.1*		*75.5*		*6.5*	*90.7*			
CR-3	TQPA %	**3.5**	1.2	2.3	**2.6**	1.1	1.5	10.7	**5.0**	0.0	**2.2**	**22.3**	2.7	**2.5**	0.7	1.7	**22.1**	16.5	**28.8**	16.5	11.4	0.3	2.7	0.89
	%*		*35.0*	*65.0*		*41.1*	*58.9*						*11.9*		*30.0*	*70.0*		*74.8*		*57.5*	*39.6*			

*relative proportions within main group (*n*-alkanes/enes, fatty acids, MAHs, PAHs, nitrogen compounds and polysaccharides). $^{+}$The aliphatic compound with mass 83+280 (likely associated to fresh OM) was not added because is not indicative of the charring effect. Total *n*-enes: total *n*-alkenes; *n*-enes >C_{18}: long-chain *n*-alkenes (>C_{18}); *n*-enes C_{18}-C_{10}: short-chain *n*-alkenes (C_{18}-C_{10}); total *n*-anes: total *n*-alkanes; *n*-anes >C_{18}: long-chain *n*-alkanes (>C_{18}); *n*-anes C_{18}-C_{10}: short-chain *n*-alkanes (C_{18}-C_{10}); other aliphatics: other aliphatic compounds (predominantly branched alkenes); Phs: phenols; total Lg: total lignin markers; total MAHs: total Monocyclic Aromatics Hydrocarbons; alkyl-B: alkyl-benzenes; total PAHs: total Polycyclic Aromatic Hydrocarbons; ITPB-PAHs: intact terpene-like plant biomass Polycyclic Aromatic Hydrocarbons; BC-PAHs: black carbon derived Polycyclic Aromatic Hydrocarbons; total N: total nitrogen compounds; BN: black carbon derived nitrogen compounds; total Ps: total polysaccharides; well pres Ps: well preserved polysaccharides; degr Ps: degraded polysaccharides; U: unidentified compounds; benz/alkyl-benz: benzene/alkyl-benzenes ratio and PAH/alkyl-PAHs: total Polycyclic Aromatic Hydrocarbons/alkylated Polycyclic Aromatic Hydrocarbons ratio. Total values of the main groups are printed in bold characters.

n-alkenes and other aliphatic compounds increased from 5.3% to 23.1% of TQPA, respectively) relative to NO-2. These changes are indicative of (i) the oxidation of an unknown proportion of the microbial SOM and the (ii) relative enrichment of aliphatic precursors, probably from degraded root components (Kaal et al., 2008b). In addition, MN-2 produced higher amounts of N-containing BC markers, as BC and BN are relatively resistant against oxidation with this reagent. The permanganate-treated MN-3 sample contained a much smaller fraction of carbohydrates than NO-3 (17.7% vs. 62.2% of TQPA), confirming that the carbohydrate-based microbial SOM is very susceptible to this oxidation agent.

The pyrolyzates of CR- samples were very different from those of NO- and MN-samples. The CR-1 sample was strongly enriched in aliphatic pyrolysis products (34% of TQPA), predominantly by regular short- chains (< C_{18}) of *n*-alkanes/*n*-alkenes and branched chain alkenes, and depleted in lignin-, carbohydrate- and *eucalyptus*- derived pyrolysis products compared with the corresponding NO-1 and MN-1 samples. The CR-1 sample produced the highest amounts of 3-ring PAHs. The benzene/alkyl-benzenes and PAH/alkyl-PAHs ratios were higher than those of NO-1 and MN-1 samples (Table 2), suggesting that a large proportion of the residual MAHs and PAHs compounds identified in the CR-1 sample could have a pyrogenic origin. A decrease of intact terpene-like plant biomass PAHs in this sample compared to sample NO-1 was also observed. These results indicate the enrichment of pyrogenic SOM in the fraction resistant to $K_2Cr_2O_7$- oxidation. Indeed, the partial resistance of BC to $K_2Cr_2O_7$ is well-documented (Knicker et al., 2007, 2008). The same studies showed the existence of a $K_2Cr_2O_7$- resistant alkyl fraction (Knicker et al., 2007, 2008), which was also supported by the pyrolyzate composition of CR-2. In fact, the increase was also observed for the *n*-fatty acids (data not shown), which are not considered part of structural aliphatic plant material. These results support that the enrichment of aliphatic material in the residual fraction of the $K_2Cr_2O_7$ oxidation residues is produced by the hydrophobic nature of these constituents (Knicker et al., 2007), possibly in combination with chemical recalcitrance of C-C bonds in methylene chains. In the CR-2 sample, $K_2Cr_2O_7$ oxidation produced a further decrease in the proportion of lignin markers (in comparison with MN-2) and microbial products such as acetamide and furans, while the BC and BN fingerprints were relatively intense (benzene,

unsubstituted PAHs, benzene carbonitriles, isoquinoline, dibenzofuran). Sample S2 also had the highest macroscopic charcoal content (Table 1). These results confirm the accumulation of BC and BN in the residues after $K_2Cr_2O_7$ oxidation. Similar to CR-1, sample CR-2 was enriched in aliphatic pyrolysis products (long- chain *n*-alkanes/*n*-alkenes, branched alkenes and intermediate *n*-alkenes). Unexpectedly, large amounts of well-preserved polysaccharides, a.o. levoglucosan, were found in samples CR-2 and CR-3. The complete depletion of fresh material in both samples suggests that cellulose fragments could be encapsulated in incompletely charred particles during fire and efficiently protected from $K_2Cr_2O_7$ oxidation. Sample CR-3 layer was also enriched in pyrogenic SOM with a high contribution of BN markers, and in an aliphatic component with particularly high contributions of branched and intermediate *n*-alkenes from an aliphatic SOM fraction, possibly in part pyrogenic, resistant to $K_2Cr_2O_7$-oxidation.

A sequential pyrolysis at 400 ºC and 750 ºC was also performed (sometimes referred to as a 'double-shot' approach) in order to distinguish between low molecular weight volatile and thermolabile SOM components and macromolecular structures in CR-2. The pyrogram produced at 400 ºC was dominated by an aliphatic fraction and carbohydrate products including levoglucosenone, while that obtained at 750 ºC was dominated by the numerous products of BC and BN (not shown). These results would suggest that a significant portion of the aliphatic material in CR-2 is not part of a macromolecular network, supporting Knicker et al. (2007) in that its chemical recalcitrance is in fact owed to inaccessibility of hydrophobic *n*-fatty acids in aqueous $K_2Cr_2O_7$ although the chemical stability of C-C bonds in linear methylene chains also would be implied in the resistance to such reagent.

3.3. Py-GC-MS: factor analysis

The first four factors (F1-F4) explained 81% of the variation in the Py-GC-MS dataset, with F1 and F2 combined accounting for 61%. The loadings of the pyrolysis products, and the scores of the samples analyzed, are shown in F1-F2 factor space (Fig. 1).

n-Alkanes/*n*-alkenes are predominantly represented in the SE quadrant with high positive loadings on F1. Higher loadings on F1-F2 were observed for the *n*-alkenes >C_{20} than for C_{10}-C_{20} *n*-alkenes. Branched alkenes and intermediate *n*-alkenes plot between their straight-chain analogues and the pyrogenic SOM markers, supporting the hypothesis that these branched aliphatics are associated with charred aliphatic precursors (Wiesenberg et al., 2009; Kaal and Rumpel, 2009).

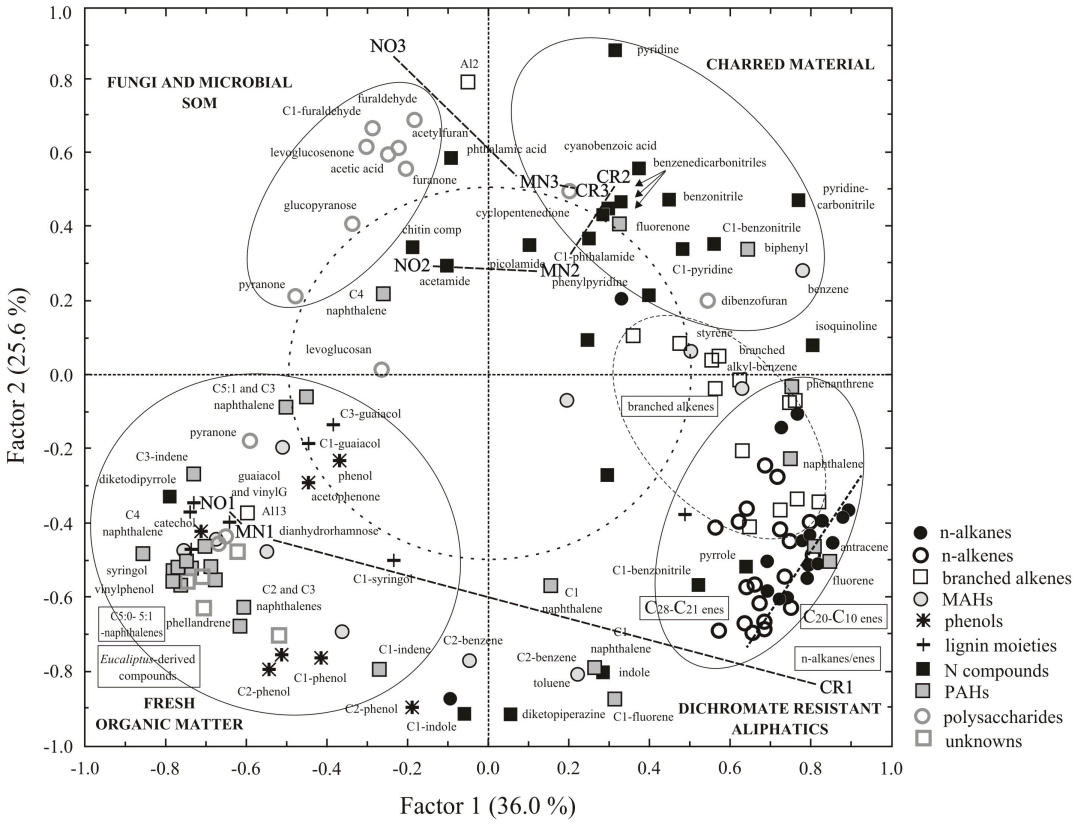

Fig. 1. Projection of the factor loadings of the pyrolysis products and sample scores in F1-F2 space. The corresponding pyrolysis product codes are given in Appendix A.

The N-containing compounds are spread throughout F1-F2 factor space, which is a result of the diverse origin of the members of this group. One cluster of N-containing compounds in the NE quadrant is composed of benzonitrile, C_1-benzonitriles, benzene dicarbonitriles, cyanobenzoic acid, pyridine, C_1-pyridine and pyridinecarbonitrile, clearly reflecting a pyrogenic origin. Indeed, many non-alkyl-substituted PAHs and dibenzofuran, also associated with BC (Pastorova et al., 1994; Naafs, 2004), plot in the same region.

The lignin-derived products (4-vinylphenol, guaiacols and syringols) and catechol occur in the SW quadrant together with an aliphatic marker of fresh plant material (Al13). Most of the remaining pyrolysis products in this region are associated with fresh or well-preserved SOM components as well: phenol and alkylphenols (in this case from lignin), $C_{5:0}$ and $C_{5:1}$ alkylnaphthalenes (from eucalyptus litter), dianhydrorhamnose (from polysaccharides) and diketodipyrrole and indoles (typical N-containing pyrolysis products of fresh proteinaceous biomass; Buurman and Roscoe, 2011). Levoglucosan plots between fresh OM and charred material; this may be attributed to the aforementioned protection of cellulose in interior parts of charcoal particles or an unknown alternative levoglucosan source. The other carbohydrate products are spread along F2 because they have multiple sources, most of which corresponding to microbial SOM. The microbial-derived polysaccharides are probably those that plot in the NW quadrant: furans (4-acetylfuran, 3/2-furaldehyde and 5-methyl-2-furaldehyde), glucopyranose, which is microbial marker (Nierop et al., 2005), and acetic acid, as this region includes unequivocal markers of chitin.

In summary, F1-F2 effectively separates the pyrolysis products according to their principal origin. Factor 1 separates the pyrogenic and aliphatic oxidation-resistant SOM fractions (chemically stable) from the fresh and degraded SOM fractions (chemically labile), while decomposed and pyrogenic SOM (strongly altered) are separated from fresh and oxidation-resistant aliphatics (resembling plant material) according to their loadings on F2.

The factor scores of the samples confirm this interpretation of F1-F2 space (Fig. 1). The samples with a large fraction of fresh biomass (NO-1 and MN-1) plot in the SW quadrant. Sample CR-1 plots in the SE quadrant, as dichromate oxidation eliminated most of the fresh

SOM causing the relative accumulation of aliphatic SOM, while BC content of this sample was negligible. The 5 ky old S2 sample is characterized by being a mixture of mainly degraded SOM and BC (with small contributions of fresh and aliphatic material). This explains why NO-2 plots in the NW region, dominated by microbial markers, while MN-2 and CR-2 plot in the NE quadrant (rich in BC markers). Sample S3 was also rich in microbial SOM but had a much lower content of chemically recalcitrant/hydrophobic aliphatic SOM (and less BC). This explains why NO-3 has a high F2 score and MN-3 and CR-3 plot in the NE quadrant reflecting BC enrichment after the removal of microbial SOM through wet oxidation. The short distance in factorial space between MN-3 and CR-3, and the large separation between these and NO-3, suggests a large and highly susceptible microbial biomass (carbohydrates, chitin) to permanganate/dichromate treatment. Thus, F1 separates samples based on the proportion of recalcitrant SOM (BC and stable aliphatic) vs. labile C while F2 separates the samples based on long-term microbial decomposition (age of SOM), e.g. fresh detritus vs. aged SOM.

Finally, based on the plotting of the sample scores in F1-F2 space, some inferences on the effects of the oxidation agents on SOM composition can be made. The minor differences between NO-1 and MN-1 can be explained by the fact that $KMnO_4$ at pH ~7.8 is a weak oxidative agent (Loginow et al., 1987; Blair et al., 1995; Tirol-Padre and Ladha, 2004) and the small microbial contribution in S1. In contrast, $K_2Cr_2O_7$ in acid medium acts as a strong oxidant (Walkley and Black, 1934), thoroughly modifying the pyrolysis fingerprint obtained from its residue (CR-1) by eliminating lignin, polysaccharides and terpenes, and relative enrichment of aliphatic and aromatic substances. In the older samples, where the aliphatic fraction is less dominant while that of BC prevails together with microbial SOM, samples treated with chemical oxidation agents (MN-2, MN-3, CR-2 and CR-3) have positive scores on F1 and F2 as both oxidants concentrate fire-derived pyrolysis products, $K_2Cr_2O_7$ again oxidizing more severely.

3.4. Solid- state ^{13}C NMR spectroscopy: results and comparison with Py-GC-MS

Samples are compared by relative intensity of the chemical shift regions. The spectrum of NO-1 (Fig. 2) was characterized by a dominant signal at 21 and a shoulder at 29 ppm

(combined 29%, Table 3) that occur in the alkyl C region, which can be ascribed to long- chain and branched aliphatic structures associated with fatty acids, lipids, waxes, cutan, suberan, cutin and suberin (Golchin et al., 1996; Kögel-Knabner et al., 1994). In the *O*-alkyl C region (45-110 ppm) a broad peak at 75 ppm (with a contribution of 29%) was detected, which is generally attributed to cellulose, hemicelluloses and pectins (Gramble et al., 1994; Kögel-Knabner, 1997). The peak at 55 ppm (9%), is likely due to methoxyl groups of lignin structures (Kögel-Knabner, 1997; Wershaw et al., 1998) but can also have contributions of *N*-alkyl from amino sugars and peptides. The *O*-substituted aromatic C between 140-160 ppm (with 3% of the relative contribution) may derive from lignin and oxidized BC. (Knicker et al., 2005a). Resonance lines of aryl C-H carbons are detected in the chemical shift region between 110 ppm and 140 ppm (14%) (Knicker and Lüdemann, 1995). The chemical shifts of carbon in carboxylic acids, esters and amides fall within the range between 160 ppm and 220 ppm and represents 15% of the total ^{13}C intensity. There are minor contributions of carbonyl or aldehydes, giving signals between 220- 245 ppm.

The NMR spectra of NO-2 and NO-3 differ considerably from that of the NO-1 sample. In NO-2, the *O*-alkyl C fraction has the highest values (26%), followed by alkyl C, carboxyl/amide C and C-H aromatic C (23%, 21% and 19%, respectively). Considering that there is no clear intensity in the phenol C region (from 160-140 ppm), this spectrum can be best explained with a considerable contribution of oxidized charcoal. The chemical oxidants applied on S1 had different effects. For the sample after extraction with $KMnO_4$ (MN-1), a spectrum similar to that of NO-1 but with higher relative intensities in the alkyl C (34%) and similar *O*-alkyl C region was acquired confirming other ^{13}C NMR studies (Tirol-Padre and Ladha, 2004) showing that cellulose seems to be resistant to permanganate oxidation. Dichromate oxidation of sample S-1 causes an increase of the relative contribution of aromatic/olefinic C (sum of C-H and COR aromatic C) (from 17% in NO-1 to 25% in CR-1) with a concomitant depletion of methoxyl C/*N*-alkyl C (from 9-4%) and *O*-alkyl C (from 29-22%).

The NMR spectrum of $K_2Cr_2O_7$ oxidized BC-rich sample (CR-2) showed the highest intensity in the chemical shift region of aromatic C (45%). Considering the absence of

methoxyl C signal, the width of the signal band (from 140-90 ppm) and the composition of the pyrolysis fingerprint, this signal most likely originates from BC (see also Skjemstad et al., 1996; Knicker et al., 2005b). Moreover, the contribution of alkyl C was strongly reduced upon $K_2Cr_2O_7$ oxidation (10% in CR-2).

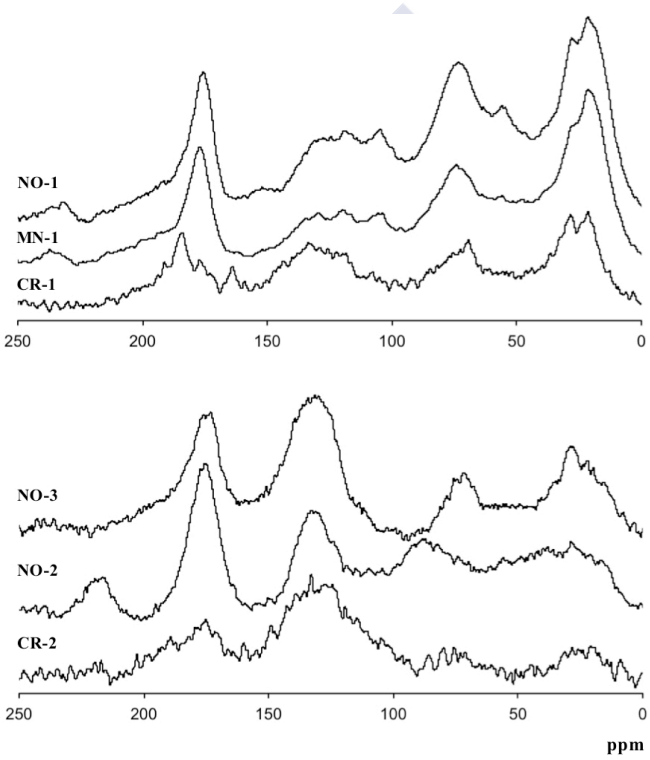

Fig. 2. Solid- state ^{13}C NMR spectra of the NaOH-extracts of untreated samples (NO-1, NO-2 and NO-3) and the NaOH-extracts of potassium permanganate and dichromate oxidized residues of S1 sample (MN-1 and CR-1) and the NaOH-extract of the potassium dichromate oxidized residues of S2 sample (CR-2).

In summary, in NO samples, ^{13}C NMR spectroscopy shows a relative decrease with depth of aliphatic C, carbohydrates and lignin moieties and a relative increase with depth of a non-lignin aromatic fraction (probably BC) and carboxyl/amide C (possibly oxidized BC in combination with N-rich microbial SOM), which is in agreement with Py-GC-MS data. With regard to the effects of chemical oxidation, both Py-GC-MS and ^{13}C NMR spectroscopies

showed a decrease of easily degradable SOM (mainly composed of fresh lignin and polysaccharides) and an increase of the aromatic fraction. Besides, $KMnO_4$ oxidation decomposes lignin only partly contrary to what has been postulated in previous studies (van Soest and Wine, 1986; Tyrol-Padre and Ladha, 2004) where lignin was strongly oxidized. The increase of aliphatic moieties upon chemical oxidation, as suggested by Py-GC-MS, was also observed by NMR spectroscopy in the superficial sample S1 although it strongly decreased in S2. However here one has to bear in mind that the alkyl C region does not only contain intensity of lipids but have considerable contributions of peptide structures or short chain alkyl groups. Those compounds are easily degraded by chemical oxidation resulting in a relative loss of the ^{13}C in the alkyl regions although lipids may have experienced a relative enrichment in the total sample.

Table 3. Chemical shift region distribution (relative proportions, %) obtained from solid-state ^{13}C NMR.

	Alkyl C (0-45 ppm)	N-alkyl C, methoxyl C (45-60 ppm)	O-alkyl C (60-110 ppm)	C-H Aromatic C (110-140 ppm)	COR Aromatic C (140-160 ppm)	Carboxyl C, amide C (160-220 ppm)	Ketone C, aldehyde C (220-245 ppm)
NO-1	29	9	29	14	3	15	1
MN-1	34	8	26	11	2	17	1
CR-1	29	4	22	19	6	20	0
NO-2	23	5	26	19	1	21	4
CR-2	10	2	16	42	3	23	4
NO-3	19	5	11	31	5	26	3

4. Conclusions

The molecular study of SOM fractions of three horizons of a colluvial soil representing ages of 100, 5000 and 9700 y was carried out before and after treatment with $KMnO_4$ and $K_2Cr_2O_7$. This provided detailed information on SOM composition (with regard to source and degradation/preservation state) and the behaviour of different SOM fractions towards these oxidation agents. *Eucalyptus*-derived terpenes and sesquiterpenes were only present in the youngest sample and resisted $KMnO_4$ but not $K_2Cr_2O_7$ treatment. Microbial biomass, recognized mainly as microbial carbohydrates (including chitin), was especially abundant in

the deeper layers of the soil and appeared highly susceptible to both $KMnO_4$ and $K_2Cr_2O_7$ treatment. As such, $KMnO_4$ and specially $K_2CR_2O_7$ concentrated two other SOM fractions abundant in this soil: straight- chain (methylene) aliphatic compounds and pyrogenic BC (with considerable amounts of N-containing functional groups). These fractions probably survived $K_2Cr_2O_7$ oxidation because of the chemical stability of polyaromatic moieties (BC) and resistant C-C bonds in methylene chains and/or hydrophobicity (the aliphatic fraction, probably root- derived). It appears that especially $K_2Cr_2O_7$ oxidation effectively concentrates BC and oxidation-resistant aliphatic structures from other SOM sources, and that in combination with Py-GC-MS it is possible to rapidly distinguish the BC while obtaining additional information on its molecular properties.

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Appendix A. Pyrolysis product list, molecular mass (M^+), fragment ions used for quantification and relative retention time to guaiacol (RT).

code	Name	M^+	mass	RT-guaiacol
10:1 - 28:1	C10-28 alkene	140 - 392	55+69	0.832 - 3.965
10:0 - 28:0	C10-28 alkane	142 - 394	57+71	0.857 - 3.969
Al1	aliphatic compound	n.d.	55+70	0.549
Al2	alkane/anal or methylated alkanol	n.d.	57+69+70	1.157
Al3	branched alkene	n.d.	55+69	1.160
Al4	branched alkene	n.d.	55+69	1.573
Al5	branched alkene	n.d.	55+69	1.590
Al6	branched alkene	n.d.	55+69	1.610
Al7	alkene	n.d.	55+69	1.901
Al8	alkene	n.d.	55+69	2.301
Al9	branched alkene	n.d.	55+69	2.428
Al10	branched alkene	n.d.	55+69	2.941
Al11	alkene	n.d.	55+69	3.069
Al12	branched alkene	n.d.	55+69	3.272
Al13	aliphatic compound	83+280	83+280	3.342
Al14	branched alkene	n.d.	55+69	3.565
Al15	branched alkene	n.d.	55+69	3.799
Ph1	phenol	94	66+94	0.782
Ph2	acetophenone	120	77+105	0.937
Ph3	C1-phenol	108	107+108	0.941
Ph4	C1-phenol	108	107+108	0.991
Ph5	C2-phenol	122	107+122	1.158
Ph6	C2-phenol	122	107+122	1.202
Ph7	catechol	110	110+64	1.348
Lg1	guaiacol	124	109+124	1.000
Lg2	4-methylguaiacol	138	123+138	1.245
Lg3	4-vinylphenol	120	120+91	1.326
Lg4	4-vinylguaiacol	150	135+150	1.519
Lg5	syringol	154	154+139	1.585
Lg6	4-methylsyringol	168	153+168	1.798
Lg7	C3-guaiacol	196	149+164	1.815
Lg8	propenoic acid, 3-(4-methoxyphenol)	178	161+178	3.296
Ar1	benzene	78	78	0.347
Ar2	toluene	92	91+92	0.431
Ar3	C2-benzene ethyl benzene	106	91+106	0.559
Ar4	C2-benzene dimethyl benzene	106	91+106	0.574
Ar5	styrene	104	78+104	0.605
Ar6	C2-benzene dimethyl benzene	106	91+106	0.612
Ar7	C3-benzene	120	105+120	0.815
Ar8	C4-benzene	134	91+119	0.887

Appendix A. (continuation)

code	Name	M^+	mass	RT-guaiacol
Ar9	C5-benzene (dimethyl-methylethyl)	148	133	1.160
Ar10	C4:1-benzene (α-dimethylstyrene)	132	132+117	1.026
Ar11	C7-benzene	176	91+92	1.662
Ar12	branched alkyl-benzene	148	91+119	1.194
Pa1	C1-indene	130	130+115	1.146
Pa2	naphthalene	128	128	1.231
Pa3	C1-naphathalene	142	141+142	1.491
Pa4	C1-naphathalene	142	141+142	1.523
Pa5	biphenyl	154	154	1.668
Pa6	C3-indene	158	143+158	1.723
Pa7	C2-naphthalene	156	141+156	1.772
Pa8	C3-naphthalene	170	155+170	2.052
Pa9	C3-naphthalene	170	155+170	2.056
Pa10	fluorene	166	165+166	2.090
Pa11	C3-naphthalene	170	155+170	2.103
Pa12	C1-fluorene	180	165+180	2.314
Pa13	9H-fluoren-9-one	180	152+180	2.353
Pa14	C4-naphthalene	184	169+184	2.382
Pa15	phenanthrene	178	178	2.442
Pa16	anthracene	178	178	2.461
Pa17	C5-naphthalene	204	183+198	2.470
Pa18	C5-naphthalene (or C2-azulene)	204	183+198	2.643
Pa19	C5:1-naphtalene	202	159+145	1.649
Pa20	C5-naphtalene	204	147+162	1.739
Pa21	C5:1-naphtalene	202	159+202	1.767
Pa22	C5-naphtalene	204	91+105	1.801
Pa23	C5-naphtalene	204	105+133	1.850
Pa24	C5-naphtalene	204	91+105	1.853
Pa25	C5-naphtalene	204	91+105	1.928
Pa26	C5-naphtalene	204	91+105	1.974
Pa27	C5-naphtalene	204	173+189	1.987
Pa28	C4-naphtalene	186	143+171	2.000
Pa29	C5:1-naphtalene	202	159+180	2.037
Pa30	C5:1-naphtalene	202	146+133	2.143
Pa31	C5:1-naphtalene	202	159+202	2.211
N1	pyridine	79	52+79	0.396
N2	pyrrole	67	67	0.402
N3	acetamide	n.d	59	0.421
N4	C1-pyrrole	81	80+81	0.501
N5	C1-pyrrole	81	80+81	0.521
N6	C1-pyridine	93	66+93	0.543
N7	benzonitrile	103	76+103	0.749
N8	pyridinecarbonitrile	104	104+77	0.887

Appendix A. (continuation)

code	Name	M^+	mass	RT-guaiacol
N9	C1-benzonitrile	117	90+117	1.011
N10	C1-benzonitrile	117	90+117	1.067
N11	1,3-benzenedicarbonitrile	128	101+128	1.302
N12	picolinamide	122	79+122	1.320
N13	1,3-benzenedicarbonitrile	128	101+128	1.322
N14	isoquinoline	129	129	1.334
N15	indole	117	90+117	1.457
N16	phthalamic acid	104	104+76	1.457
N17	1,3-benzenedicarbonitrile	128	101+128	1.463
N18	C1-indole	131	130+131	1.664
N19	C1-pthalimide	161	161+76	1.719
N20	cyanobenzoic acid	147	76+147	1.827
N21	phenylpyridine	155	154+155	1.833
N22	chitin-derived compound	167	125+167	1.883
N23	diketodipyrrole	186	93+186	2.280
N24	diketopiperazine compound	194	70+194	2.646
Ps1	acetic acid	60	60	0.203
Ps2	furanone compound	84	54+84	0.434
Ps3	3/2-furaldehyde	96	95+96	0.483
Ps4	acetylfuran	110	95+110	0.613
Ps5	5-methyl-2-furaldehyde	110	109+110	0.686
Ps6	4-hydroxy-5,6-dihydro-(2H)-pyranone	114	58+114	0.755
Ps7	dianhydrorhamnose	128	113+128	0.861
Ps8	cyclopentenedione compound	112	69+112	0.869
Ps9	levoglucosenone	126	68+98	0.989
Ps10	3-hydroxy-2-methyl-4H-pyran-4-one	126	71+126	1.046
Ps11	dianhydro-alpha,D-glucopyranose	144	57+69	1.263
Ps12	dibenzofuran	168	168+139	1.953
Ps13	levoglucosan	162	60+73	2.123
U1	alpha phellandrene	136	91+93+136	0.827
U2	U2 (possibly methylcyclohexane)	96	67+96	0.710
U3	U3	157	117+157	1.871
U4	U4	200	185+200	2.186
U5	U5	212	197+202	2.247
U6	U6	203	203	3.152

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CHAPTER 4

The impact of shrimp farm effluents on the soil carbon storage and the geochemistry of mangrove soils under semi-arid climate conditions in northern Brazil

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List of authors:

M. SUÁREZ-ABELENDA[a], T.O. FERREIRA[b], M. CAMPS-ARBESTAIN[C], V.H. RIVERA-MONROY[d], F. MACÍAS[a], G.N. NÓBREGA[b], X.L., OTERO[a]

[a]Departamento de Edafoloxía e Química Agrícola, Facultade de Bioloxía, Universidade de Santiago de Compostela, 15782- Santiago de Compostela, Spain.

[b]Departamento de Ciencias do Solo, Centro de Ciencias Agrarias, Universidade Federal do Ceará, Fortaleza, Brazil.

[c]Institute of Natural Resources, Private Bag 11222, Massey University, Palmerston North 4442, New Zealand.

[d]Department of Oceanography and Coastal Sciences, School of the Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, USA

The impact of shrimp farm effluents on the soil carbon storage and the geochemistry of mangrove soils under semi-arid climate conditions in northern Brazil

Summary

A semi-arid mangrove-dominated - mainly by *Rhizophora* and *Avicennia sps.* - estuary system in the Northeast Brazilian coast (Ceará state) was selected for this study. The area has a marked seasonality with 8-mo y^{-1} of intense drought. The main objective of the study was to determine the impact of shrimp farm wastewater effluents (which occupy 26.5% of the study area) on the geochemistry and the organic carbon (OC) storage of these soils and estimate the total amount of OC stored in soils of semi-arid mangrove estuary systems. Wastewater-affected mangrove was referred to as WAM and undisturbed areas as non-WAM. Redox conditions and OC content were found to be statistically related ($P<0.05$) with seasonality and type of land use (WAM vs. non-WAM). Eh values oscillated from anoxic to oxic conditions (from –5 to 68 mV in WAM and from <40 to >400 mV in non-WAM soils) in the wet season and significantly higher ($P <0.01$) in the dry season (from 66 to 411 mV). OC contents fluctuated between 1.9-1.1 and 1.4-1.1 kg m^{-2} in the wet and dry season, respectively, for non-WAM, and between 0.8-0.6 and 0.6-0.4 kg m^{-2} in the wet and dry season, respectively, for WAM soils. Iron partitioning was significantly dependent ($P<0.05$) on type of land use, with a smaller degree of pyritization (mean values of 7% vs. 44%) and lower Fe-pyrite presence (mean values of 10 μmol g^{-1} vs. 61 μmol g^{-1}) in WAM soils compared to non-WAM soils. Basal respiration of soil sediments was significantly influenced ($P<0.01$) by type of land use with highest CO_2 evolution rates detected in the WAM soils (mean values of 0.20 mg CO_2 h^{-1}–g^{-1} C vs. 0.04 mg CO_2 h^{-1}–g^{-1} C). We hypothesized that the decrease in OC storage in WAM soils is mainly due to (i) an increase in microbial activity caused by the loading of rich-nutrient effluents and (ii) by an increase of strong electron acceptors [e.g., NO_3^-], that promotes a decrease in pyrite and thus in that of soil OC burial. Stocks of OC in the first 40 cm depth of the semi-arid mangroves from Ceará State (area of 22,936 ha) are estimated to be ~1.5 million t.

1. Introduction

Wetlands are characterized by their capacity to stabilize high amounts of organic matter (IPCC, 2007) at the global scale. Out of the 1500 Gt of organic carbon (OC) accumulated in the pedosphere (Lal, 2001) about 250 Gt are preserved in tropical wetlands (Neue et al., 1997). It is estimated that worldwide mangroves accumulate 1,023 Mg C ha^{-1} (Donato et al., 2011) and are considered potential sinks of "blue carbon", which is the term used to refer to carbon accumulated in sea-grass meadows, mangrove forests and tidal salt marshes (Chmura et al., 2003; Duarte and Cebrián, 1996; Duarte et al., 2005; Bouillon et al., 2008). There are numerous estimates of the amount of OC stored in mangroves in tropical and subtropical latitudes. However, only few incorporate the contribution of mangrove wetlands in semi-arid regions (Giani et al., 1996). Rates of OC burial in mangrove ecosystems range from 1.39 to 6.54 t C ha^{-1} yr^{-1} (Duarte and Cebrián, 1996; Duarte et al., 2005; Bouillon et al., 2008) and the total amount of OC stored in worldwide mangrove sediments ranges from 4 to 20 Pg (Donato et al., 2011).

The surface area of tropical wetlands is estimated in 5 million km^2 (Neue et al., 1997) while that of mangroves is 137,760 km^2 distributed in 118 countries and territories (Giri et al., 2011). The second largest area of mangrove forest in tropical and sub-tropical latitudes is harboured in Brazil (9,627 km^2; occupying the 7% of the total global extension), only overcome by Indonesia (with 31,130 km^2). Brazil has the largest mangrove area in South America but this is currently being cut back due to (i) human settlements, (ii) conversion to agriculture and silviculture, and (iii) the establishment of aquaculture shellfish farms in the intertidal areas where mangrove is the dominant vegetation. This has caused the loss of 46% of Brazil mangrove area since 1983 (IBAMA, 2005). For example, to present shrimp farming has caused the destruction of the 26.5% of mangrove forest in the Ceará State (Northeast Brazilian coast) (IBAMA, 2005).

The decline in the mangrove area entails a considerable economic loss since mangrove ecological services support other economic-driven activities (e.g. estuaries and offshore fisheries) in coastal areas. On average, the current price of one hectare of mangroves ranges

between US$ 9,900-37,500 (Constanza et al., 1997, Aburto-Oropeza et al., 2008). Mangrove ecosystems have a critical role as a nutrient filter in the intertidal zone due to its efficient capacity to process excess N and P loads resulting from human practices, e.g. shrimp aquaculture and agriculture/urban development (Páez-Osuna et al., 1998; Marchand et al., 2011). Shrimp farming production on the tropical coastline has increased lately, caused by its high economic value, which is estimated to range between US$200,000 and 900,000 ha^{-1} (UNEP-WCMC, 2006). However, these estimates do not include the ecological cost associated to the conversion of mangrove habitats to shrimp farms (Páez-Osuna, 2001). Nonetheless, it is now recognized that this type of conversion tends to be unsustainable at the long term (Rivera-Monroy et al., 1999; Primavera et al., 2007), thus negatively affecting both local inhabitants and their environment (Marchand et al., 2011). Therefore, new management strategies should be developed so that these types of activities become sustainable and compatible with the maintenance of natural resources in mangroves. Moreover remediation actions allowing the recovery of degraded areas should be put in place.

Regular flooding on mangrove ecosystems reduces the O_2 flux through the sediments and, consequently, anoxic conditions are generated. In the absence of O_2, other species (i.e. NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-} and CO_2) are used as electron acceptors (Froelich et al., 1979) by anaerobic microorganisms (Berner, 1984) following a sequential pattern. In mangrove anoxic environments the reduction of Fe(III) and sulphate are the main processes involved in the oxidation of OC to CO_2 (Huerta-Diaz and Reimer, 2010). Fe(II) and hydrogen sulphide are concurrently formed; the former by Fe^{+3} (crystalline Fe-oxyhydroxides) bacterial reduction and the latter by either SO_4^{2-} reduction or through SOM decomposition (both mediated by anaerobic bacteria). A metastable fraction (known as acid volatile sulphides, AVS) is formed being the latter transformed into pyrite, which is the stable form under anoxic conditions (Berner, 1970; 1984). The availability of dissolved OC, temperature regime and sedimentation rates have an important role in this process (Huerta-Diaz and Reimer, 2011). When changes in hydrology and/or human activities decrease the overlying water column thickness, sub-superficial conditions in the sediment may become suboxic and even oxic. Under oxic conditions, the oxidation-reduction potential Eh increases and pyrite is oxidized due to its

thermodynamic instability under such conditions –although this reaction can be further catabolized by bacteria– generating SO_4^{2-} and acidity (Otero and Macías, 2002). The value of pH will lower when the acid neutralizing capacity of the soil is surpassed (van Breemen and Buurman, 2002). Factors such as roots density, extent of the hydroperiod and bioturbation by macrofauna also affect O_2 diffusion and thus pyrite oxidation (Otero and Macías, 2002; Ferreira et al., 2007). Therefore, the understanding of the spatial variability that controls the Eh regime in mangrove soils as a result of human activity (e.g. shrimp effluents) is critical to understand the C and nutrient cycling under such conditions.

The objective of this research was to acquire an in-depth understanding of how soil C storage in mangrove wetlands from semi-arid region is affected by shrimp farm wastewater effluents. We hypothesized that (i) the release of rich-nutrient waters and its effects on the biological activity, and (ii) climatic seasonality influence soil redox conditions by increasing the concentration of strong electron acceptors [e.g. NO_3^-], and this has an effect on the pyritization rate of sediments and, as a result, on that of soil C storage.

2. Materials and Methods

2.1. Description of the area under study and soil sampling

The study was carried out in two sites: (i) the Aracati estuary ("Baixo Jaguaribe") and (ii) the estuary of Acaraú. Both estuaries are located in the state of Ceará (Brazil), the former at the southeast and the latter at the northwest. These sites were selected as representative of semi-arid mangrove-dominated estuary systems in the north eastern Brazilian coast (Figure 1). The mangrove area in Ceará state is 22936 ha (Menezes, 2005). The region has a semi-arid warm-tropical climate, with a mean annual temperature of 26-28ºC and mean annual precipitation of 982-1130 mm (IBGE, 1999). Soil moisture and temperature regime are classified as "peraquic-hiperthermic" (USDA, 2006); soil sediments are classified as thionic fluvisols (IUSS Working Group WRB, 2006) or sulfaquents (Soil Taxonomy, 2006); the mangrove forest is dominated by *Rhizophora mangle* L. and *Avicennia schaueriana* L.

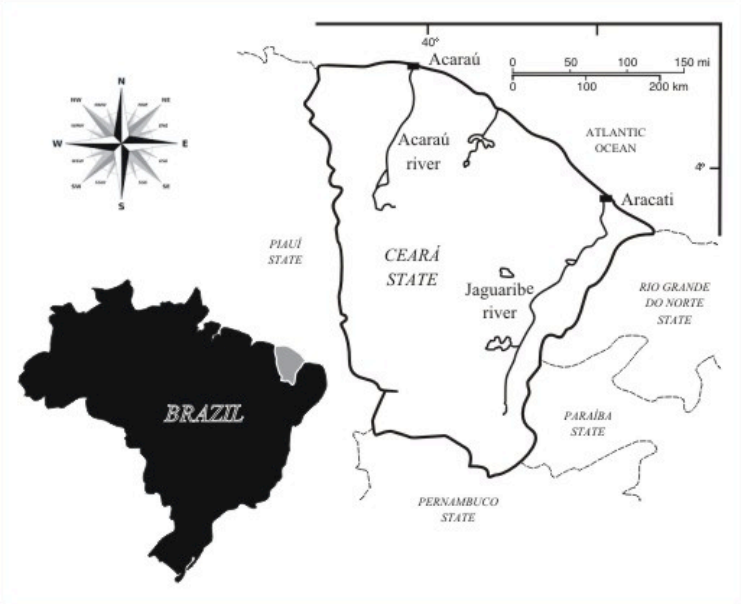

Figure 1. Location of the study area.

Soil samples were collected during the rainy season (January-April) and the dry season (May-December) in 2010. The selected sites were obtained in (i) areas without human disturbance (referred to as non-WAM – "non Wastewater Affected Mangroves") and (ii) areas severely affected by wastewater effluents derived from shrimp farming activities (referred to as WAM – "Wastewater Affected Mangroves). WAM samples were sampled in the Acaraú estuary. They were taken at a distance of 100 m from the effluent emissary of a shrimp farm that begun its activity in 2001. In both estuaries, non-WAM samples were taken in areas where there was no evidence of disturbance. The total number of sampling sites selected was fifteen. Seven of them were taken in well-preserved areas under *Rhizophora mangle* L. (during both dry and wet season), three in well-preserved areas under *Avicennia schaueriana* L. (wet season) and five in areas affected by shrimp farming under *Rhizophora mangle* L. (wet season). Data corresponding to *Avicennia* areas were only used for the carbon stock assessment. At each sampling site, three replicates were collected from intertidal sediments at low tide using a 50 cm long PVC core tubes (5 cm i.d.). Previous studies determined large concentrations of

nutrients in wastewaters discharges released from aquaculture farms in the Ceará state. Values of 6.4 and 0.8 mg l^{-1} of NH_4^+ and PO_4^{3-} in solution, respectively, were recorded by Figueiredo et al. (2005) in shrimp farms from the Jaguaribe basin. These correspond to 29.4 and 3.0 kg ha^{-1} y^{-1} of NH_4^+-N and PO_4^{3-}-P, respectively.

2.2. Analytical methods

2.2.1. pH, redox measurements and bulk density

pH and Eh were measured *in situ*; pH was measured using a glass electrode (model MP120, Mettler Toledo), previously calibrated with pH 4.0 and 7.0 standards, and Eh with an oxidation-reduction potential (ORP) platinum electrode after an equilibration time of several minutes. Final Eh readings were corrected to refer it to a calomel reference electrode by adding 244 mV. The core tubes were sealed on-site and kept at approximately 4 °C while being transported to the laboratory in a vertical position. The PVC sediment cores were cut into sections (0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm), dried at 60º until constant weight, and bulk density was determined in triplicate (values were 0.35±0.03, 0.42±0.10 0.51±0.01, 0.48±0.01 for *Rhizophora sp.* and 0.37±0.03, 0.50±0.05, 0.54±0.06 and 0.47±0.01 g cm^{-3} for *Avicennia sp.*).

2.2.2. Organic carbon determination, Fe partitioning and AVS.

Dried samples were sieved to 2 mm and then treated with 0.5 N HCl to eliminate carbonates, although later analysis confirmed the lack of inorganic C in these sediments. HCl-treated sediments were then carefully washed with distilled H_2O and total OC was measured using a CNS analyser (LECO Induction Furnace instruments) once the samples were air-dried. For samples collected in the wet season, the sequential extraction of Fe was performed by a combination of the methods described by Huerta-Díaz and Morse (1990) and Raiswell et al. (1994). This approach discriminates oxy-hydroxide-Fe (extracted with sodium citrate + sodium bicarbonate + sodium dithionite) from pyrite-Fe (extracted with concentrated HNO_3). Before the extraction of pyrite-Fe, samples were treated with 10 N HF for 16 h under agitation to

eliminate Fe in silicate sheets and with concentrated H_2SO_4 thereafter, to eliminate Fe associated with organic matter. AVS were determined according to the method of Kostka and Luther (1994). Sulfide from AVS was liberated with 1 N HCl during 40-50 min under a continuous flow of N_2. The evolved H_2S was trapped in Zn acetate (3%) and precipitated as ZnS. Sulfide was then measured colorimetrically (670 nm). A detailed description of the aforementioned methods and the degree of Fe pyritization calculation is found in Ferreira et al. (2007).

2.2.3. CO_2 evolution measurement

25 g of fresh soil sediment of WAM and non-WAM samples collected during the wet season at different depths (0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm) were used to study the short term evolution of CO_2 under aerobic conditions using a Micro-Oxymax Respirometer (Columbus Instruments, Columbus, OH). The instrument contains a set of gas sensors that allow the continuous measurement of CO_2 concentration in the headspace of the reaction vessels. The moisture content of the soil sediments was adjusted to the specific field capacity of each soil and the light opening was controlled to minimize its effects on soil respiration. Incubation was carried out at 25 °C during 12 days.

2.3. Carbon stock determination

The C content was estimated down to 40 cm and reported as kg C m^{-2}. The calculated stock was based on measurements of elemental C content (g C kg^{-1}soil) (after the removal of CO_3-C) and bulk density (g cm^{-3}) values, which were determined for each depth interval. In the carbon stock assessment, the "shrimp wastewater affected area" (Table 2) was estimated taking into account that (i) in the previous records no substantial changes in the OC content were detected in areas farther than 100 meters from the effluents, and (ii) that the mean area of each shrimp farm in the Ceará state is approximately of 20 ha (and assuming a square shape) (Rocha and Rodrigues, 2001), plus considering the aforementioned minimum distance of 100 m, gives an area of approximately 18 ha; that multiplying by the total number of farms in the Ceará (83 according to Rocha and Rodrigues, 2001) gives a total area of ~1,500 ha. The so-called "well-

preserved mangrove area" (~19,800 ha) was determined from difference between the total mangrove area (22,936 ha – SDR, 2005) and the sum of the area occupied by shrimp farms (1,619 ha – Rocha and Rodrigues, 2001) and the shrimp wastewater affected area.

2.4. Statistical analysis

Statistical differences in soil physicochemical properties between (i) the two types of land use, (ii) the two distinct climatic seasons, and (iii) the different soil layers were determined using a factorial ANOVA. All statistical analyses were carried out using SPSS computer software. Degree of significance between fixed variables and dependent variables are described in Table 1.

Table 1. Factorial ANOVA analysis. Significances between fixed variables and dependent variables.

	pH	Eh	Corg	Fe-pyrite	Fe-oxyhydroxides	AVS	CO_2 accumulated
PERIOD	**	*	**	n.d.	n.d	n.d.	n.d
USE	**	n.s	**	*	**	n.s.	**
DEPTH	n.s	n.s	**	n.s.	n.s.	n.s.	n.s.

**: highly significative (P<0.01); *: significative (P<0.05); n.s.: not significative (P>0.05); n.d: not determined

3. Results

3.1. Redox potential and pH

Significant differences (P<0.05) in Eh values were observed between wet and dry seasons, but not between types of soil use (i.e. non-WAM vs. WAM samples) or among layers (P>0.05) (Table 1). During the wet season, Eh values ranged between –5 and 68 mV in WAM soils (Figure 2) indicative of a slightly anoxic environment. Soils not affected by discharge (non-WAM) have typical values reported for mangroves soils with oxidized conditions (>400 mV) in the most superficial layer (0-10 cm depth) and reduced conditions (value <40 mV) below 10 cm depth (Figure 2). During the dry season, the Eh values of WAM and non-WAM

soils increased with values significantly higher (P<0.05) than samples collected during wet season, ranging between 66 to 411 mV. The results thus indicate that the system is well buffered against Eh changes given that as the redox potential remains within a narrow range.

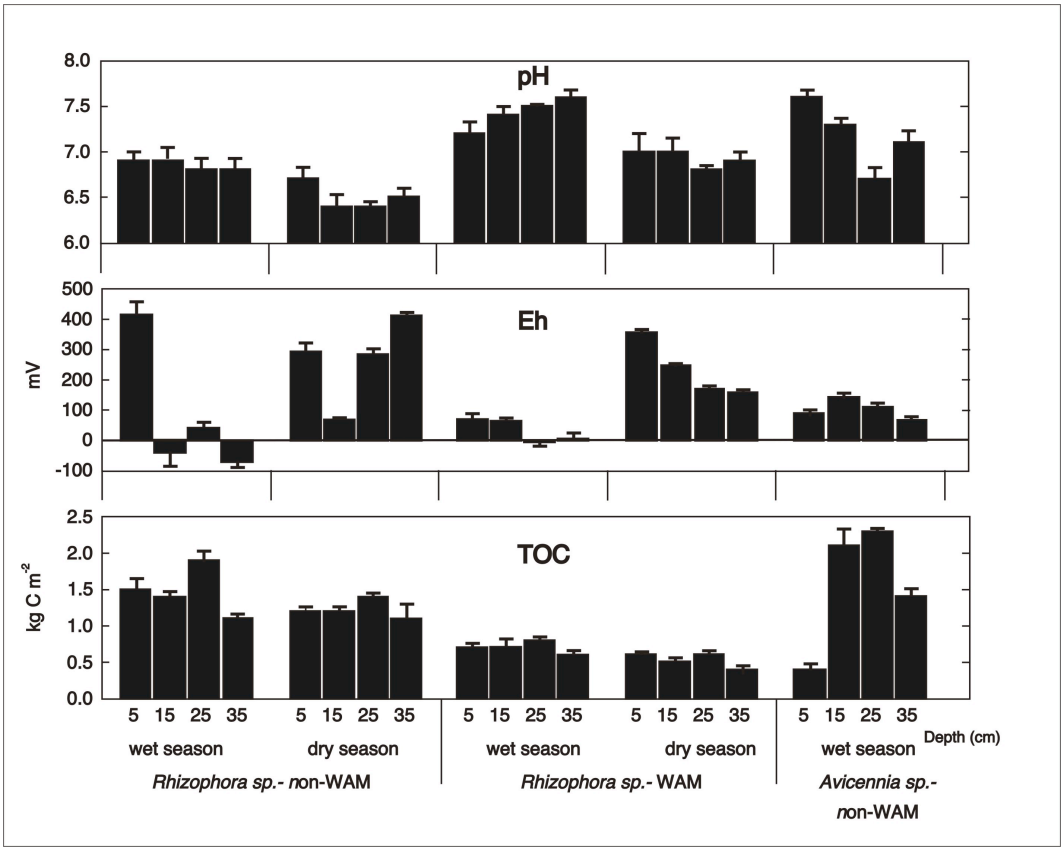

Figure 2. pH and redox measurements, and OC content in (i) non-WAM (non-Wastewater Affected Mangrove) soils and WAM (Wastewater Affected Mangroves) soils at (ii) different seasonal periods, and (iii) under *Rhizophora sp.* and *Avicennia sp.* stands.

Significant differences (P<0.01) in pH values were found among seasons and among types of soil use (Table 1). In the wet season, non-WAM soil pH values were ~6.9 and significantly (P<0.01) lower than those in WAM soils, which ranged between 7.2 and 7.6 (in topsoil and 40-cm depth, respectively) (Figure 2). In the dry season, pH values were significantly lower (P<0.01) than those of the wet season, and those of in non-WAM soils

(range 6.4-6.7) were significantly lower ($P<0.01$) than those in WAM soils (range 7.0-6.8) (see Figure 2).

3.2. Iron (Fe) partitioning

Fe-partitioning was studied during the wet season and results indicate the existence of a different geochemistry depending on the type of soil use (Figure 3 A-B). Fe-associated with pyrite concentration values in WAM soils ranged from 5.5 to 17.7 μmol g^{-1} and were significantly lower ($P<0.05$) than those non-WAM soils (values ranging from 22.5 to 94.0 μmol g^{-1}). In non-WAM soils, this fraction was found to be greatest in the deepest soils layers (30-40 cm) (94.0 μmol g^{-1}) followed by the intermediate layers, with the lowest value in the superficial layer (22.5 μmol g^{-1}). In the WAM soils, these values ranged from 17.7 to 5.5 μmol g^{-1}, in shallow and deepest layers, respectively. The Fe concentration values associated with Fe oxy-hydroxides in WAM soils were significantly higher ($P<0.01$) than those in non-WAM soils. In non-WAM soils, the deepest layer displayed the highest value (106.1 μmol g^{-1}) while in overlaying layers values ranged from 54.5 to 69.9 μmol g^{-1} (values for layer 0-10 cm depth and layer 20-30 cm depth, respectively). In WAM soils, the Fe associated with Fe oxy-hydroxides presented similar contents in all layers (values ranging between 130.9 to 142.2 μmol g^{-1}) (Figure 3).

3.3. Organic carbon content and short-term CO_2 evolution

In mangrove forests dominated by *Rhizophora sp.,* significative differences ($P<0.01$) in OC content were observed among seasons, land uses and soil layers (Table 1). Thus, the highest values were found during the wet season in soils not affected by shrimp effluents (non-WAM soils) with values ranging from 1.9 to 1.1 kg m^{-2} (in 20-30 and 30-40 cm depth, respectively) while in the dry period these values decrease (values ranging between 1.4 and 1.1 kg m^{-2}, Figure 2). The OC stocks of WAM soils during wet season are significantly lower ($P<0.01$) with mean values ranging from 0.8 to 0.6 kg m^{-2} while in the dry season mean values ranged from 0.6 to 0.4 kg m^{-2}. In the *Avicennia sp.* dominated forest, the highest OC stocks was observed at 20-30 cm depth with mean values of 2.3 kg m^{-2} and lower values of 0.4 kg m^{-2} in

the topsoil. The % of OC in non-WAM soils oscillated from 5.9 to 3.1 and from 4.8 to 3.0 in wet and dry season, respectively while in WAM soils fluctuated from 2.9 to 1.8 and from 2.4 and 1.2 in wet and dry season, respectively, under *Rhizophora sp.* stands. In non-WAM soils under *Avicennia sp.* the OC percentage ranged between 6.5 and 1.5 during the wet season.

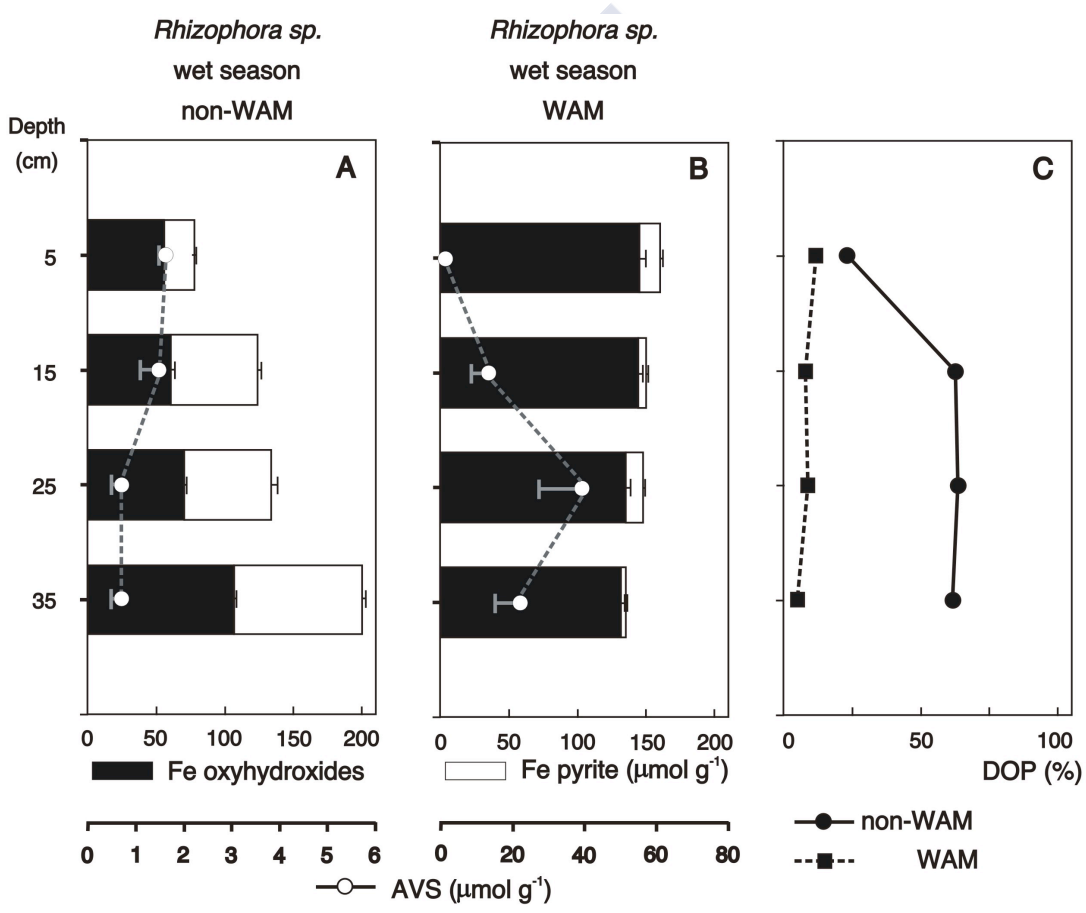

Figure 3. Iron partitioning, acid volatile sulphides and degree of pyritization in non-WAM soils and WAM soils under *Rhizophora sp.* sampled in the wet season.

Basal respiration of the top 10 cm layer of WAM soils was significantly higher ($P<0.01$) than the corresponding top 10 cm layer in the non-WAM soils (means: 0.414±0.130 and 0.116±0.058 mg CO_2 h^{-1}–g^{-1} C in shallow layers, respectively) (Figure 4). WAM soils presented the highest rates of CO_2 released in deeper layers (0.466±0.192 mg CO_2 h^{-1}–g^{-1} C in layer 20-30 cm depth) although no significant differences ($P>0.05$) were found between layers. Accumulated values of released CO_2 per g of C during 12 days of incubation are provided in Figure 5. Total CO_2 emitted from samples taken at 20-30 cm depth was seven times higher in WAM soils than non-WAM soils (values of 67.1 and 8.8 mg CO_2-g^{-1} C in 12 days, respectively).

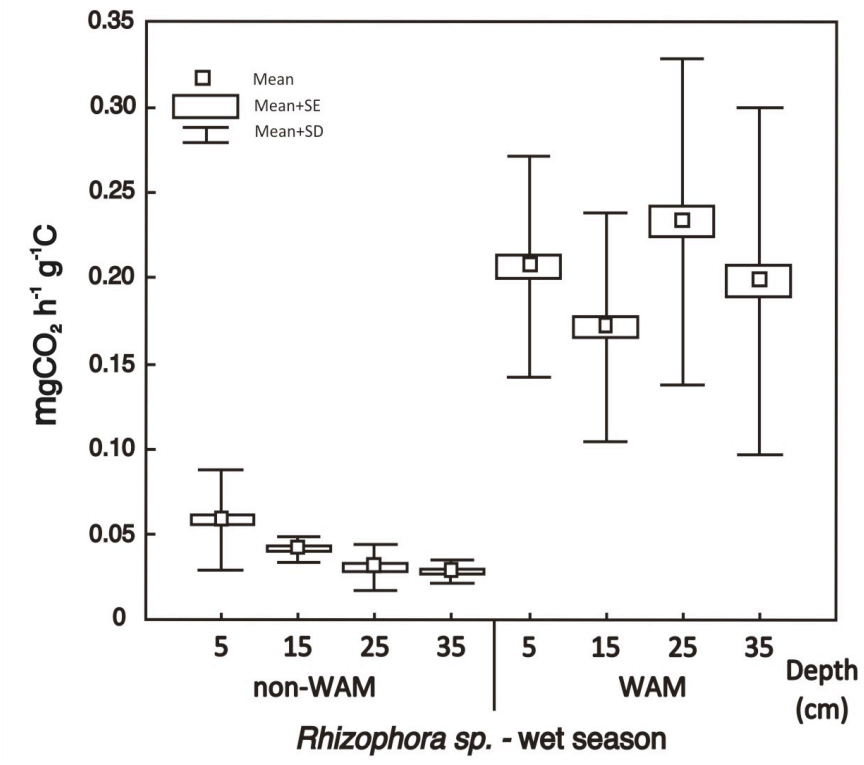

Figure 4. Carbon dioxide emission rate (mg CO_2-C h^{-1} g^{-1}) in non-WAM soils and WAM soils under *Rhizophora sp.* stands sampled in the wet season.

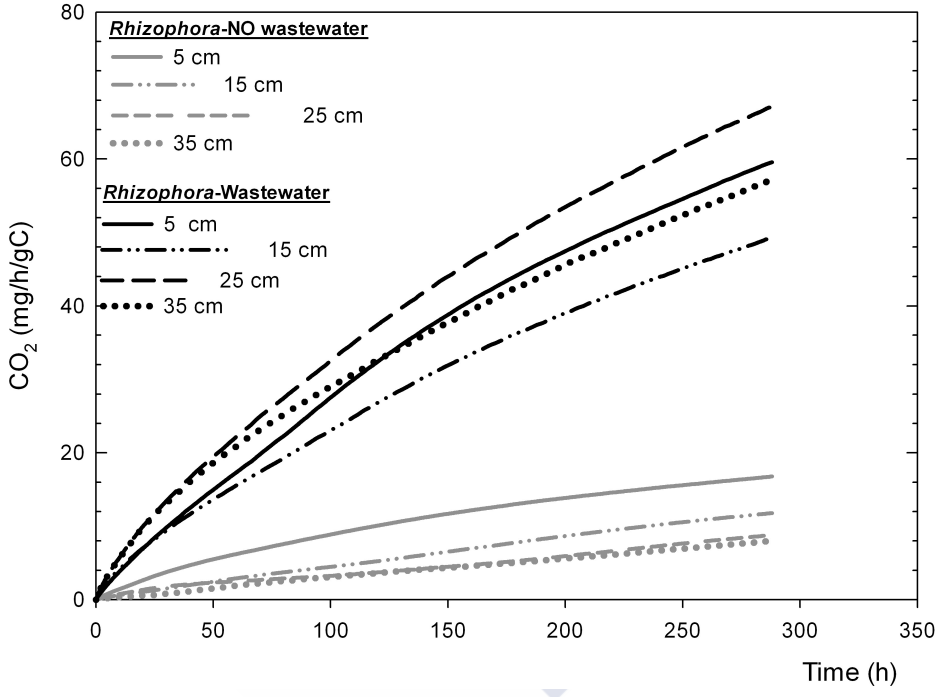

Figure 5. Cumulative CO_2 emission during the 12 days of incubation under oxidized conditions of non-WAM soils and WAM soils under *Rhizophora sp.* sampled at different depths during the wet season.

4. Discussion

4.1. Variation in the interstitial water: pH and Eh

Mangrove soils development and properties are influenced by temporary or permanent water saturation within the upper sediment layers. This study evidences that the biogeochemical properties of these soils change as result of differences in hydrological conditions between seasons, as reflected in the observed redox values (Twilley et al., 1992; Marchand et al., 2011).

The Eh values of WAM soils during the wet season (mean value of 68 mV in the top 10 cm of soil) were indicative of the existence of a slightly anoxic environment (<100 mV

according to Ferreira et al., 2010). The discharge of effluents affects the hydroperiod –duration of inundation and depth– and this has been reported to be particularly evident close to the discharge point where it led to a decrease in redox potential (Marchand et al., 2011). The top soils of areas not affected by discharge, e.g., non-WAM soils, had typical values as reported for mangroves soils with oxidant values (mean value of 435 mV in the top 10 cm of soil). Nonetheless, it should be noted that differences among soil uses were not significant at $P<0.05$. During the dry season, characterised by low precipitation and high evapotranspiration (IBGE, 1999), the Eh values of WAM and non-WAM soils were in the suboxic range for soils with neutral pH (Eh between 100-300 mV; Ferreira et al., 2010). During this period, there was an increase in soil water loss that led to the exposure of superficial layers in most instances and, therefore, to oxic conditions (Otero and Macías, 2002, Marchand et al., 2011). It should be noted that, an increase in pH only, with no change in Eh, displaces the elements to a more oxidised state (Pourbaix, 1974).

Redox conditions fluctuations trigger changes in acid-base conditions in mangrove substrates. The fact that pH values during dry season were slightly but significantly lower than those in measured in the rainy period (e.g. top soil of non-WAM: in the wet season 6.9; in dry season 6.7) were associated to the higher Eh values of the former (Figure 2). This was attributed to the oxidation of reduced compounds (NH_3, H_2S, FeS_2) and the associated release of protons (Otero et al., 2006). The decrease in pH was however minor compared to other studies with clear pattern of seasonal variation (pH values <5 for high salt marsh in summer; Otero and Macias, 2002), which suggests a pH-buffering response in studied mangroves. Values of pH in WAM soils are considerably higher than in non-WAM soils (top 10 cm in wet season: 7.2 and 6.9, respectively); this was attributed to the inputs from shrimp farming effluents rich in base cations, resulting in pH values close to 7 or slightly higher.

4.2. Effect of shrimp farm wastewater on the Fe geochemistry and nutrient inputs

In mangroves, pyrite is the main fraction of free Fe especially at depth, where it is stable in the absence of air and in presence of dissolved sulphide (Berner, 1970). In non-WAM soils, the degree of pyritization was around 50% (Figure 3C), which indicates that the amount of

reactive Fe available for sedimentary pyrite formation (Berner, 1984) is large enough to support this process, especially down to 10 cm layer. In these soils, the low amounts of AVS (Figure 3A) are in agreement with a thermodynamically stable condition for pyrite formation where the most of the metastable Fe sulphides are evolved to pyrite (Berner, 1970).

Although it is known that seasonal changes can affect soil redox processes (Ferreira et al., 2007), it is clear the extremely low degree of pyritization observed in WAM soils was caused by the anthropic land use change. Specifically, it was attributable to the high NO_3^- input from the wastewater to the interstitial water. Previous studies highlight high concentrations of nutrients from shrimp farming discharges, N being dominant (Páez-Osuna et al., 1998). Nitrogen is added to shrimp ponds as feed being incorporated into animal tissue or metabolized into NH_4^+ and later transformed into NO_3^- via NO_2^- by bacterial nitrification. Briggs et al. (1994) determined that only the 24% of the N applied into the ponds in the form of feed is retained by shrimps whilst the 35% is discharged to the effluent water and the 31% remains in the sediments. In quantitative terms, it was estimated that the discharge from shrimp ponds to coastal waters in Mexico were 2866 t N y^{-1} and 462 t P y^{-1} (Páez-Osuna et al., 1998).

We hypothetize that the low values of Fe-pyritic in WAM soils are caused by (i) the presence of NO_3^-, which acts as electron acceptor in the bacterial-mediated oxidation of pyrite (Schippers and Jorgensen, 2002), along with the Fe^{2+} released in the reaction (equation 1), and (ii) the concomitant inhibition of Fe (III) and sulphate reduction. In fact, it has recently been observed the role of NO_3^- as mediator of pyrite oxidation (Schippers and Jorgensen, 2002; Jørgensen et al., 2009). Studies under *in-vitro* conditions have shown that the microbial oxidation of pyrite with the use of NO_3^- as electron acceptor results in the subsequent formation of SO_4^{2-} (Jørgensen et al., 2009) (equation 1). Jørgensen et al. (2009) observed a 2-3-fold increase in the NO_3^- reduction when pyrite added to soil. This process is associated with an increase in pH, as protons are consumed in the reaction. Nitrate may also interfere the formation of FeS and decrease dissolved Fe^{2+} thereby inhibiting pyrite formation. Thus, nitrate acts as electron acceptor in the microbial oxidation of dissolved FeS promoting the SO_4^{2-} release and formation of Fe(III) hydroxide precipitates (Schippers and Jorgensen, 2002)

(equation 2). The dissolved Fe^{2+} that is implicated in the Fe-sulphides/pyrite formation can thereafter react with NO_3^- and lead to the formation of Fe(III) hydroxide precipitates (Jørgensen et al., 2009) (equation 3). The first reaction will counteract part of the increase in acidity associated to reaction 2 and 3.

$$5FeS_{2(s)} + 14NO_3^-{}_{(aq)} + 4H^+{}_{(aq)} \Rightarrow 7N_{2(g)} + 10SO_4^{2-}{}_{(aq)} + 5Fe^{2+}{}_{(aq)} + 2H_2O_{(aq)} \quad (1)$$

$$5FeS_{(s)} + 9NO_3^-{}_{(aq)} + 8H_2O_{(aq)} \Rightarrow 5Fe(OH)_{3(s)} + 5SO_4^{2-} + 9/2\ N_{2(g)} + 9H^+{}_{(aq)} \quad (2)$$

$$5Fe^{2+}{}_{(aq)} + NO_3^-{}_{(aq)} + 12H_20_{(aq)} \Rightarrow 5Fe(OH)_{3(s)} + 1/2\ N_{2(g)} + 9H^+{}_{(aq)} \quad (3)$$

Theses aforementioned processes promote the decrease of pyrite in the system, along with that of OC that is likely associated to the burial of SOM (Berner and Raiswell, 1983; Huerta-Diaz and Reimer, 2010). In recent sediments (or when there is a change in the geochemical conditions) with an active process of pyrite formation/oxidation, sulphate reduction entails a SOM oxidation. However in mature sediments, where pyrite is consolidated and sulphate is consumed at depth, OC becomes established. Adsorption of organic molecules to the surface of pyrite (with positive net charge when pH is below 6.8) by weak ionic bonds would also explain a partial OC stabilization (Pontes-Buarques et al., 2001). It should be noted that the shrimp farm activity of the study area involves the discharge of wastewaters from ponds every three months, so the impact occurs at determined periods magnifying their consequences.

4.3. Stock of organic carbon in mangrove soils

4.3.1. Organic carbon content and CO_2 evolution associated to seasonal variation and shrimp farm discharge

Leptosols, planosols and luvisols (IUSS Working Group WRB, 2006) are dominant in the northeast steppe region (Brazilian Caating) (Romero and Ferreira, 2010) and are characterized by their extremely low amounts of organic matter (Bernoux et al., 2002). Mangrove soils of the study area show a high OC content (values >4%) when compared with

surroundings soils (<0.6%) and larger than the mangrove global median value of 2.2% (Kristensen et al., 2008).

The increased rainfall during the wet season can affect pore water salinity, redox potential, and pH, along with soil biogeochemical processes (Alongi et al., 1999, 2004), and therefore affect the C cycle. In the dry season, when the water flux is low, the presence of O_2 in sediments increases with the consequent increase in SOM oxidation associated to aerobic respiration. During this period however, enhanced deposition of litter and semi-decomposed material along with dissolved OC in superficial layers caused by the low hydric dynamic efficiency (Twilley et al., 1992) may contribute to increase the OC stocks. The results obtained in this study indicates that the C storage capacity of the soils were considerable smaller in the WAM soils compared with the non-WAM soils. The addition of nutrients along with the wastewater discharge increased belowground microbial activity (Feller et al., 1999) promoting the decomposition of organic matter of these oligotrophic soils. Therefore the releasing of nutrients from shrimp ponds to areas of mangroves may increase the microbial activity and organic matter decomposition. Besides NO_3^- may acts as oxidant of the organic matter in the absence or under low O_2 concentrations, further accelerating this process.

Cumulative basal respiration of the non-WAM soils after 12 d incubation was below 17 mg C-CO_2 g^{-1} total soil C and decreased with depth, whereas in the WAM soils the highest values were found in the 20-30 cm depth layer followed with that at the surface, with values always above 67 mg C-CO_2 g^{-1} C. The high values of accumulated CO_2 released from WAM soils compared with the non-WAM can be associated to (i) the presence of a high fraction of available SOM caused by a lower physico-chemical protection by mineral fraction against microbial attack as compared with that of the burial effect of SOM and OC–Fe-pyrite surface interactions or/and (ii) a high content of nutrients –associated to the incorporation of rich nutrients wastewaters– that were limiting microbial growth under natural conditions. An increment of SOM mineralization will cause a greater contribution to CO_2 emissions to atmosphere and a concomitant decrease of C stocks. Therefore the capacity of mangrove soils to act as CO_2 sinks will become reduced (Bouillon et al., 2008).

Table 2. Estimation of C stoked in Ceará State mangroves and CO_2 emission rate down to 40 cm depth. Comparative with others studies.

		estimation of C stoked (down to 40 cm depth)	
	season	kg C m^{-2}	t OC/mangrove area
non-WAM soils under Rhizophora	wet	8.1	
	dry	6.7	
non-WAM soils under Avicennia	wet	8.7	
WAM soils under Rhizophora	wet	3.8	
	dry	2.9	
accumulated OC in well-preserved mangrove areas			1462581
accumulated OC in shrimp farm effluent-affected mangrove areas			50618
released OC by direct implementation of shrimp farms (mangrove destruction)			119453
total OC released (losses by mangrove destruction plus losses by effluents effecs)			179066
total OC stock in mangroves of Ceará state			1513198
area occupied by mangrove in Ceará State (ha)			22936
area occupied by well-preserved mangroves in Ceará State (ha)			19823
area occupied by shrimp farms in Ceará mangrove (ha)			1619
shrimp wastewater affected area (ha)			1500
authors	localization, depth		kg C m^{-2}
Donato et al. (2011).	Indo-Pacific, 30 cm		22.0
Vegas-Vilarrúbia et al. (2010).	Lower Orinoco Delta, 1 m		22.5
Matsui (1998)	Australia, Hinchinbrook Channel, 50 cm		29.6

According to *Secretaria de Políticas de Desenvolvimento Regional, (SDR), Brasil., 2005; **Rocha and Rodrigues, 2001

4.3.2. Carbon stock assessment

In mangrove forest dominated by *Rhizophora sp.* the highest C content was found in non-WAM soils during the wet season with mean values of 6.0 kg C m^{-2}. Values of 2.8 kg C m^{-2} were determined for WAM soils. In *Avicennia sp.* mangrove forest a slightly higher value, 6.2 kg C m^{-2}, was recorded for the same period (Table 2). The calculated OC stored in the organic layers for the well-preserved mangroves down to 40 cm depth was estimate to be slightly greater than 1.45 million t of C, whereas the calculated OC stored in shrimp farm wastewater affected-mangrove areas was estimate to be larger than 50,000 t C (Table 2). Thus, the total OC stocked in the studied mangroves was estimated to be above 1.5 million t of C. The OC losses by aquacultural activities were estimated around 179,000 t C based on the evaluation of (i) the losses by mangrove destruction (direct implementation of ponds and buildings) and (ii) that caused by the nutrient-rich effluents from shrimp farm wastewaters. After ~9 y of releasing wastewater effluents, the calculated CO_2 emission rate was estimated in 4.4 t C ha^{-1} y^{-1}. A number of studies have estimated C stocks in mangrove sediments. Donato et al. (2011) reported mean values of C storage of approximately 22.0 kg C m^{-2} in mangrove soils down to 30 cm depth from the Indo-Pacific coast; Vegas-Vilarrubia et al. (2010) reported mean values of 22.5 kg C m^{-2} down to 1 m depth in the lower Orinoco Delta; finally, mean values of 29.6 kg C m^{-2} down to 50 cm depth were found in mangroves from the Hinchinbrook Chanel (Australia) by Matsui (1998) (Table 2).

5. Conclusions

The study in the mangroves of Ceará has enabled us to (i) determinate how shrimp farming negatively affects soil OC accumulation, with special attention at the effect of wastewater effluents, and (ii) make an estimation of organic C accumulated in the system. To present, there is a considerable awareness of the expansive activity of aquaculture in this region as well as the implications it has on the destruction of a vast area of riverine mangrove; however, no evidences of the effects of the contaminated wastewater on OC from mangrove-dominated estuaries were provided. According to this study, rich-nutrient effluents from shrimp ponds could increase the microbial activity in soil sediments and thereby decrease the OC

content. Besides a large NO_3^- concentration in wastewaters may be involved in the bacterial-mediated oxidation of pyrite (as we support through the sequential extraction of Fe). In stable anoxic conditions, high concentrations of pyrite are accompanied by high concentrations of buried organic matter. When these conditions are modified, i.e. increase of Eh values or the aforementioned effect of NO_3^-, pyrite is degraded with the concomitant decay of OC. We conclude that the studied mangroves have a high potential capacity to store C, despite of being subject to semiarid climate conditions, but they are highly susceptible to human practices. Large efforts are need to understand how changes in biogeochemical process affect to OC dynamics in sediments of mangroves and make the shrimp farming a sustainable activity. This will allow the conservation of biogeochemical conditions of mangrove and maintain their environmental features, while having economic revenue. As in agriculture, aquaculture systems need to take into account their role as C pool and preserve their environmental functions.

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CHAPTER 5

Final conclusions

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FINAL CONCLUSIONS

In this thesis the following research areas have been investigated: (i) the chemistry of SOM in acid soils that differ in their degree of SOM saturation with Al, (ii) the susceptibility of SOM to oxidation by chemical reagents of different reactivity ($K_2Cr_2O_7$ and $KMnO_4$) and (iii) the biogeochemical processes involved on SOM stabilization in hydromorphic soils under mangroves and the impact of the discharge of shrimp farming wastewater on these.

The conclusions obtained in each of these three areas of research are summarized as follows:

1) Comparison of the mechanisms involved in the stabilization of SOM in (i) soils developed from highly-weatherable materials and (ii) those from low-weatherable rocks under similar temperate climatic conditions.

1a. Acidic and highly dystrophic soils developed on materials poor in weatherable minerals, such as quartzites under humid temperate conditions and good drainage, tend to accumulate primary SOM at the surface horizon as conditions are not adequate for microbial activity.

1b. Acidic but eutrophic soils developed on materials rich in weatherable minerals, such as basalt under perudic conditions and good drainage at early stages of development (e.g., soils with andic properties), promote an ideal environment for fresh SOM decay.

1c. In these soils (1.b), secondary SOM (e.g., microbially-derived) is highly preserved, this being the dominant fraction of SOM. Therefore, under the specific pedogenic conditions such as those of soils with andic properties, once primary SOM has been decomposed under initial "ideal conditions" for decomposition, "non-ideal conditions" for decomposition of secondary SOM emerge. The literature attributes this to (i) interaction with reactive Al surfaces forming micro-aggregates (Eusterhues et al., 2003; *Organic Geochemistry* 34, 1591-1600.), and (ii) the generation of local anoxic circumstances inside these micro-aggregates inhibiting SOM decay (Buurman et al., 2007; *European Journal of Soil Science* 58, 1330-1347). No

evidence of the existence of a specific recalcitrant fraction in primary SOM was found in this study.

2) SOM resistance againts potassium dichromate ($K_2Cr_2O_7$) and potassium permanganate ($KMnO_4$) oxidation in a BC-rich Haplic Umbrisol representing ages of ca. 100, 5000 and 9700 y old under different vegetation.

2a. Fresh SOM-derived compounds such terpenes and sesquiterpenes present in *Eucalyptus sp.* litter were only detected in the soil sampled at the surface (e.g., 5-10 cm depth) and thus relatively young (<150 y old). These compounds were found to be stable against the oxidation with $KMnO_4$ but not with acid $K_2Cr_2O_7$ under heat.

2b. Microbial biomass, recognized mainly identified by the presence of microbial carbohydrates, was especially abundant in the sample taken at 190-195 cm depth (~9,700 y old) and appeared shown to be highly susceptible to both $KMnO_4$ and $K_2Cr_2O_7$ treatment.

2c. $KMnO_4$ promoted the oxidation of carbohydrate products, mostly from (i) microbial SOM but also from (ii) the lignocellulose fraction, causing a relative enrichment of aliphatic moieties and aromatic black C structures.

2d. acid $K_2Cr_2O_7$ oxidation under heat concentrated straight-chain/methylene aliphatic compounds and pyrogenic BC with considerable amounts of N-containing black C markers. These fractions probably resisted such oxidation because of the chemical stability of BC-derived polyaromatic structures and resistant C-C bonds in methylene chains and/or hydrophobicity of aliphatic moieties probably root-derived.

2e. acid $K_2Cr_2O_7$ oxidation under heat in combination with Py-GC/MS enables a rapid identification of BC and provides additional information on its molecular properties.

3) **SOM stabilization in hydromorphic soils of semiarid mangrove systems and effect of rich-nutrients wastewaters in the geochemistry.**

3a. Rich-nutrient effluents from shrimp ponds were shown to increase the microbial activity of soil sediments nearby and thereby decrease the OC content.

3b. Large NO_3^- concentration (a strong electron acceptor in the absence of O_2), in wastewaters from shrimp ponds may be involved in the bacterial-mediated oxidation of pyrite. In these ecosystems, the latter is generally found associated with high concentrations of buried SOM under stable geochemical conditions. When these conditions were modified, i.e. the aforementioned load of NO_3^--rich waters, pyrite was degraded with the concomitant decay of OC.

3d. The mangroves under study have a high potential capacity to store C, despite of being under semiarid climate conditions; however, they are fragil ecosystems highly susceptible to disruption with unsustainable human practices.

3e. Large efforts are need to understand how changes in the biogeochemistry of these systems affect the OC dynamics and ensure that the sustainability of shrimp farming practices. This will allow the preservation of the natural biogeochemical conditions of mangroves and maintain their environmental features, while having economic revenue. As in agriculture, aquaculture systems need to take into account their role as a C pool and preserve their environmental functions.

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APPENDIX 1

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In Appendix 1.1 (page 209 onwards) illustrations of pyrograms studied in the chapter 2 and obtained from NaOH-extracted residues of $K_2Cr_2O_7$ and $KMnO_4$ oxidation as well as non-oxidized samples of a black C-rich soil are provide. In addition some of the most representative pyrolysates (represented structures) are included next. NO-1: toluene (*m/z* 91+92), guaiacol (m/z 109+124), 4-vinyl guaiacol (*m/z* 135+150), $C_{4:1}$ naphthalene compound (e.g. *m/z* 146+133), C_4-naphthalene (*m/z* 169+194), hexadecanoic acid (C_{16} *n*-fatty acid) and octadecanoic acid (C_{18} *n*-fatty acid) (*m/z* 60+73), and branched alkene (*m/z* 55+69). MN-1: toluene (*m/z* 91+92), methyl phenol (*m/z* 107+108), 2-ethyl 3,4-dimethyl phenol (*m/z* 107+122), $C_{4:1}$ naphthalene compound (e.g. *m/z* 159+202), levoglucosan (*m/z* 60+73), C_3-naphthalene (*m/z* 155+170), C_{16} *n*-fatty acid and C_{18} *n*-fatty acid (*m/z* 60+73), and series of pairs of *n*-alkanes/alkenes (*m/z* 57+71 and 55+69, respectively) (*). CR-1: benzonitrile (*m/z* 76+103), ethyl phenol (*m/z* 107+122), naphthalene (*m/z* 128), biphenyl (*m/z* 154), fluorene (*m/z* 165+166) and regular series of pairs of *n*-alkanes/alkenes (*m/z* 57+71 and 55+69, respectively) (*) and *n*-fatty acids (*m/z* 60+73) (†). NO-2: acetic acid (*m/z* 60), methyl furaldehyde (*m/z* 95+96), phenol (*m/z* 66+94), methyl phenol (*m/z* 107+108), methyl benzonitrile (*m/z* 90+117), methyl indene (*m/z* 130+115), biphenyl (*m/z* 154), cyanobenzoic acid (*m/z* 76+147), diketodipyrrol (*m/z* 93+186) and sterol compound (preg-4-ene 3,20-dione compound) (*m/z* 121+299). MN-2: branched alkenes (*m/z* 55+69), resorcinol (*m/z* 54+110), benzonitrile (*m/z* 76+103), phenyl pyridine (*m/z* 154+155), C_2 naphthalene (*m/z* 141+156), C_{16} *n*-fatty acid and C_{18} *n*-fatty acid (*m/z* 60+73) and regular series of pairs of *n*-alkanes/alkenes (*m/z* 57+71 and 55+69, respectively) (*). CR-2: pyridine carbonitrile (*m/z* 104+77), methyl benzonitrile (*m/z* 90+117), benzenedicarbodinitrile (*m/z* 101+128), picolinamide (*m/z* 79+122), anthracene (*m/z* 178), methyl fluorene (*m/z* 165+180), cyanobenzoic acid (*m/z* 76+147), isoquinoline (*m/z* 129), C_{16} *n*-fatty acid and C_{18} *n*-fatty acid (*m/z* 60+73) and regular series of pairs of *n*-alkanes/alkenes (*m/z* 57+71 and 55+69, respectively) (*). NO-3: acetic acid (*m/z* 60), furaldehyde (*m/z* 95+96), methyl furaldehyde (*m/z* 95+96), levoglucosenone (*m/z* 68+98), dianhydro-alpha, D-glucopyranose (*m/z* 57+69), C_4-naphthalene (*m/z* 169+184), methoxy cinnamic acid (*m/z* 161+178) and C_{16} *n*-fatty acid (*m/z* 60+73). MN-3: pyridine (*m/z* 52+79), pyrrole (*m/z* 67), styrene (*m/z* 78+104), benzonitrile (*m/z* 76+103), benzenedicarbodinitrile (*m/z* 101+128), cyanobenzoic acid (*m/z* 76+147) and C_{16} *n*-fatty acid and C_{18} *n*-fatty acid (*m/z* 60+73). CR-3: methyl pyridine (*m/z* 66+93), picolinamide (*m/z* 79+122), biphenyl (*m/z* 154), C_{16} *n*-fatty acid and C_{18} *n*-fatty acid (*m/z* 60+73) and a set of unidentified compounds.

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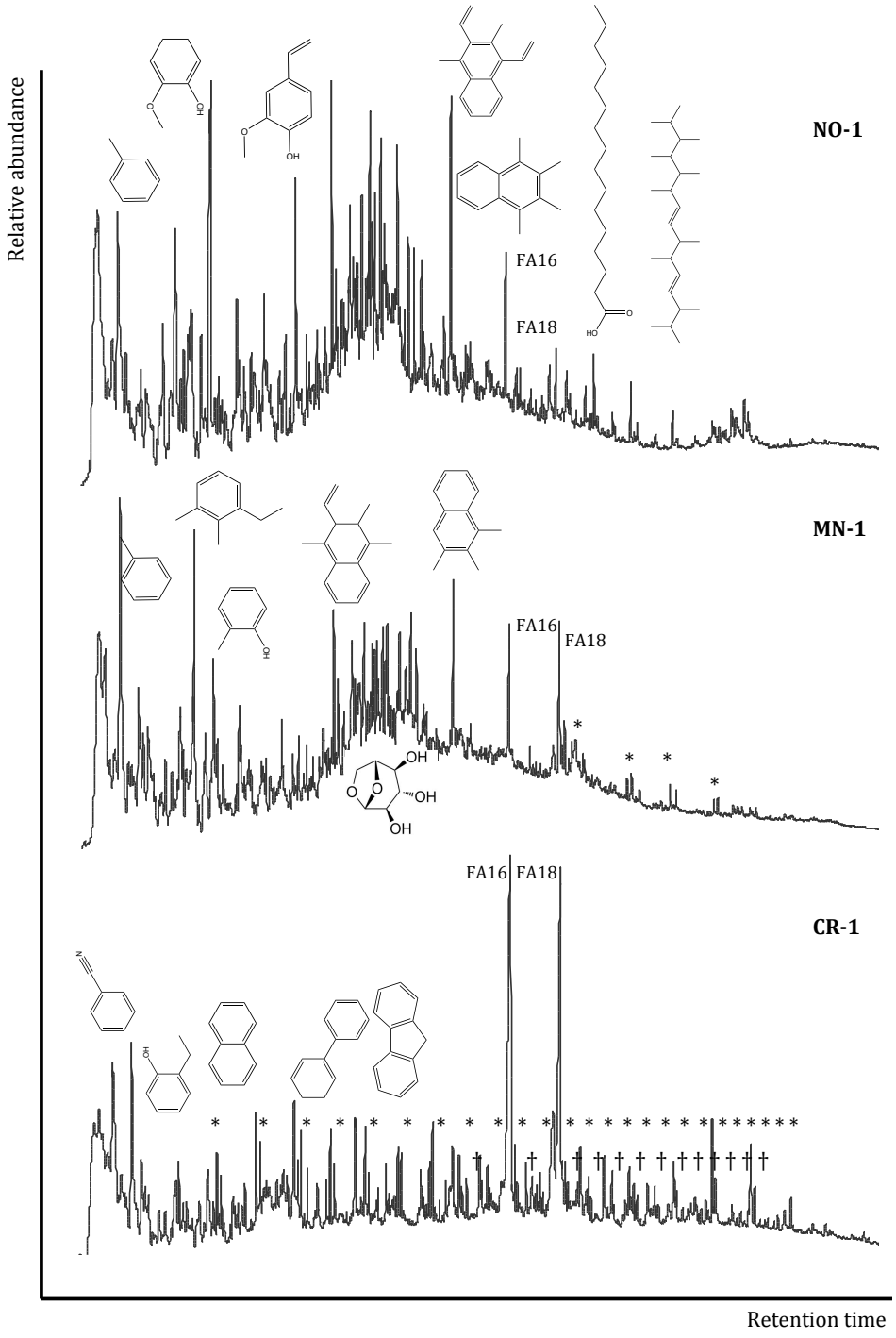
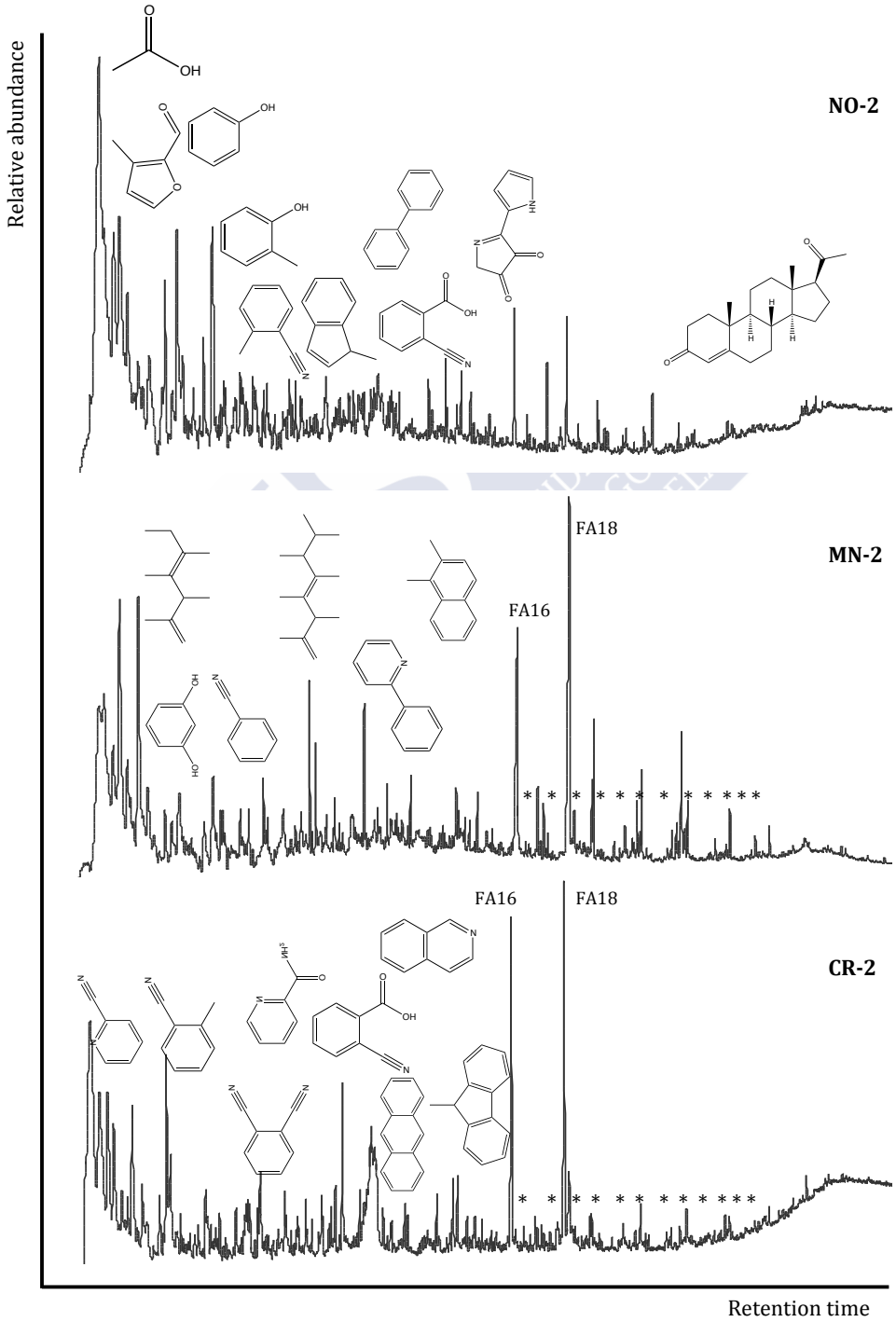
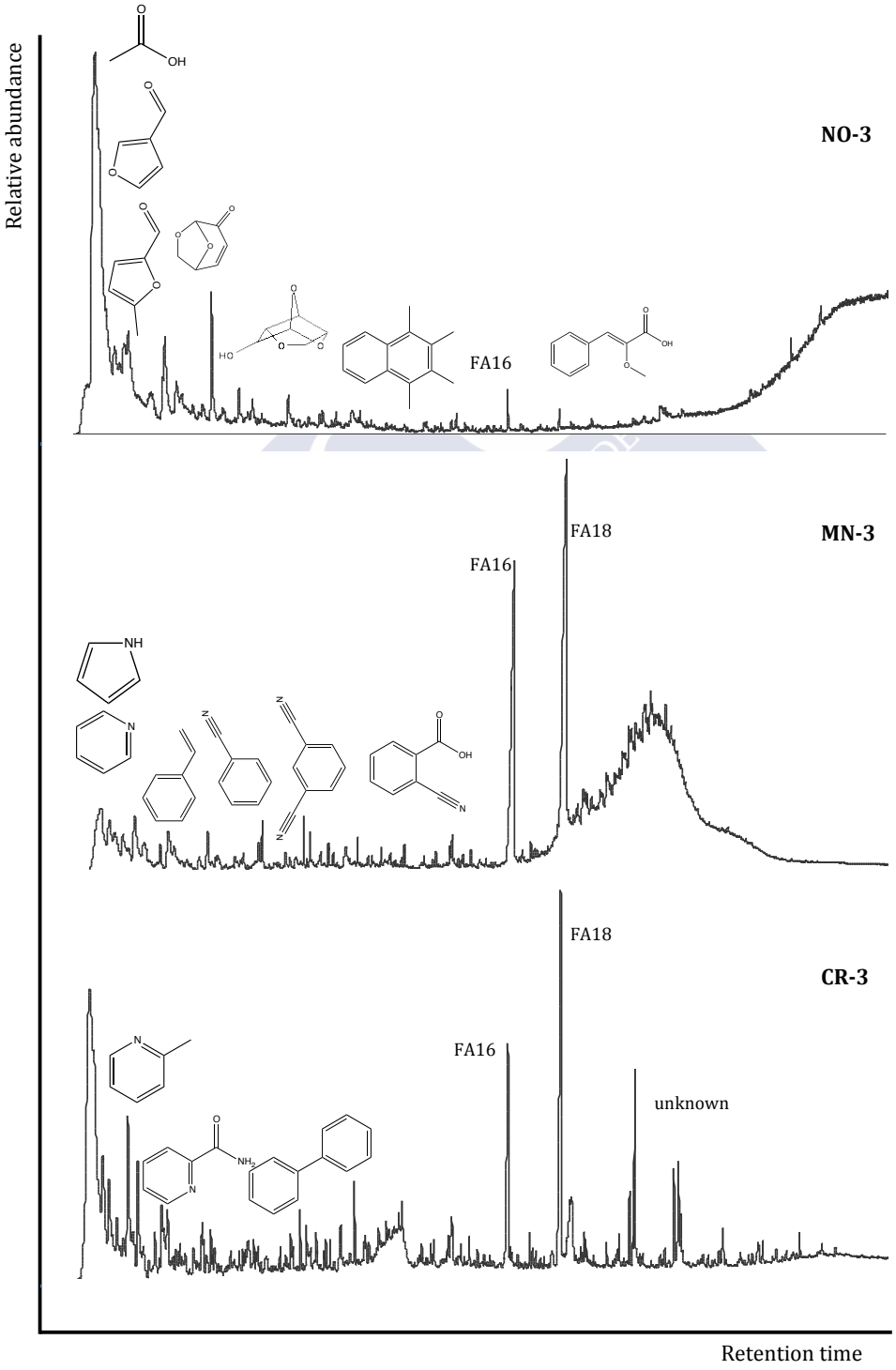
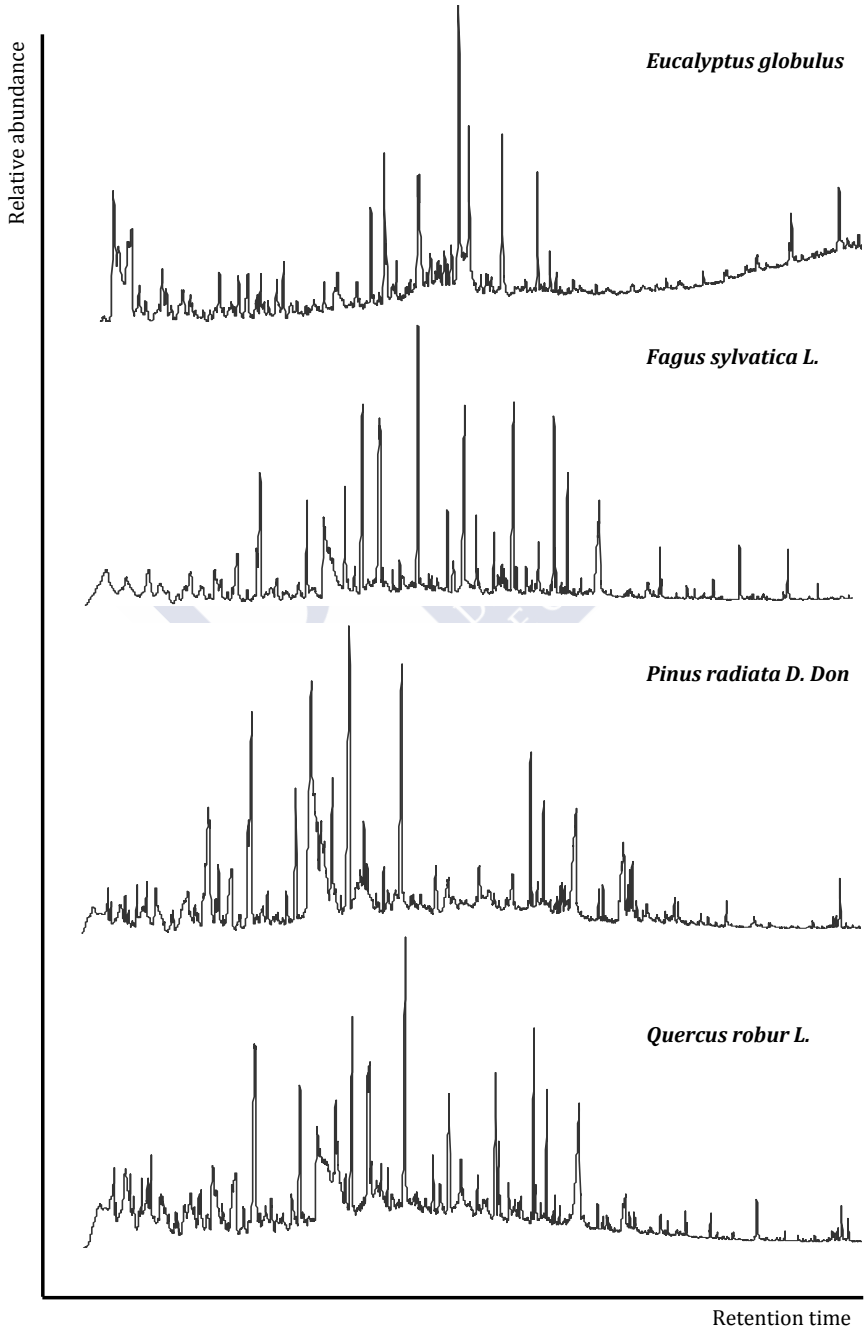

Relative abundance
NO-1
FA16
FA18
MN-1
FA16
FA18
CR-1
FA16
FA18
Retention time

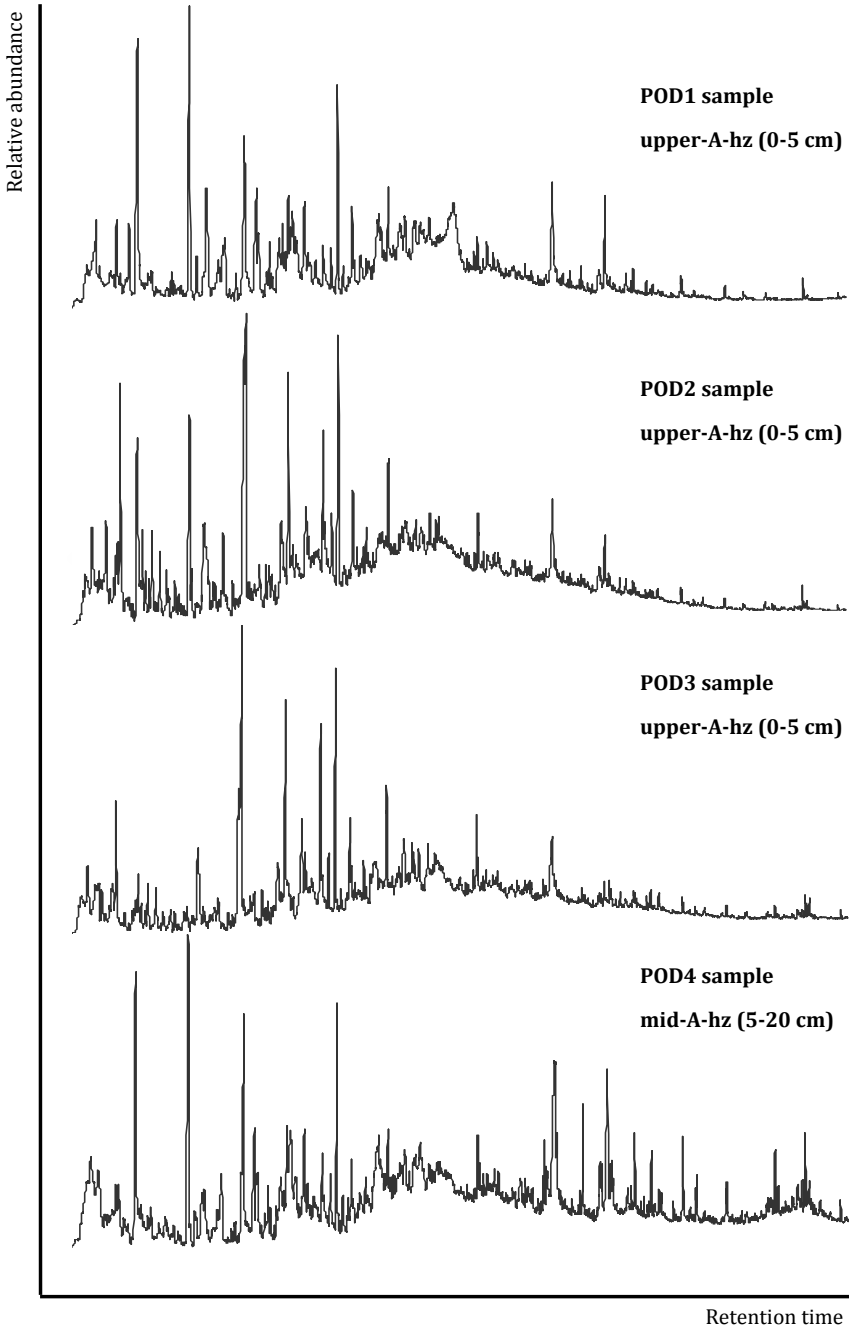

Relative abundance
NO-2
FA18
FA16
MN-2
FA16
FA18
CR-2
Retention time

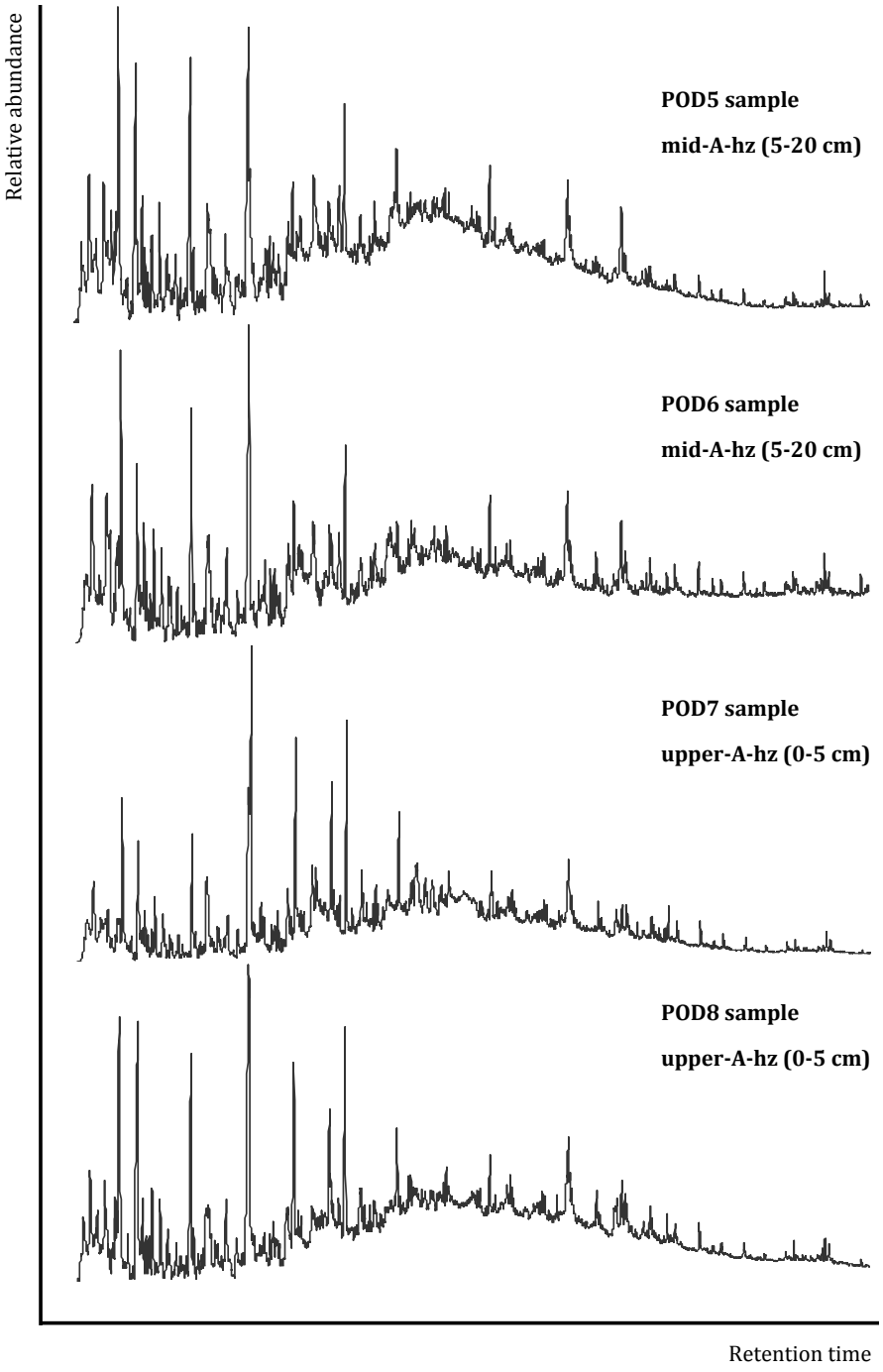

Relative abundance
NO-3
FA16
MN-3
FA18
FA16
CR-3
FA18
FA16
unknown
Retention time

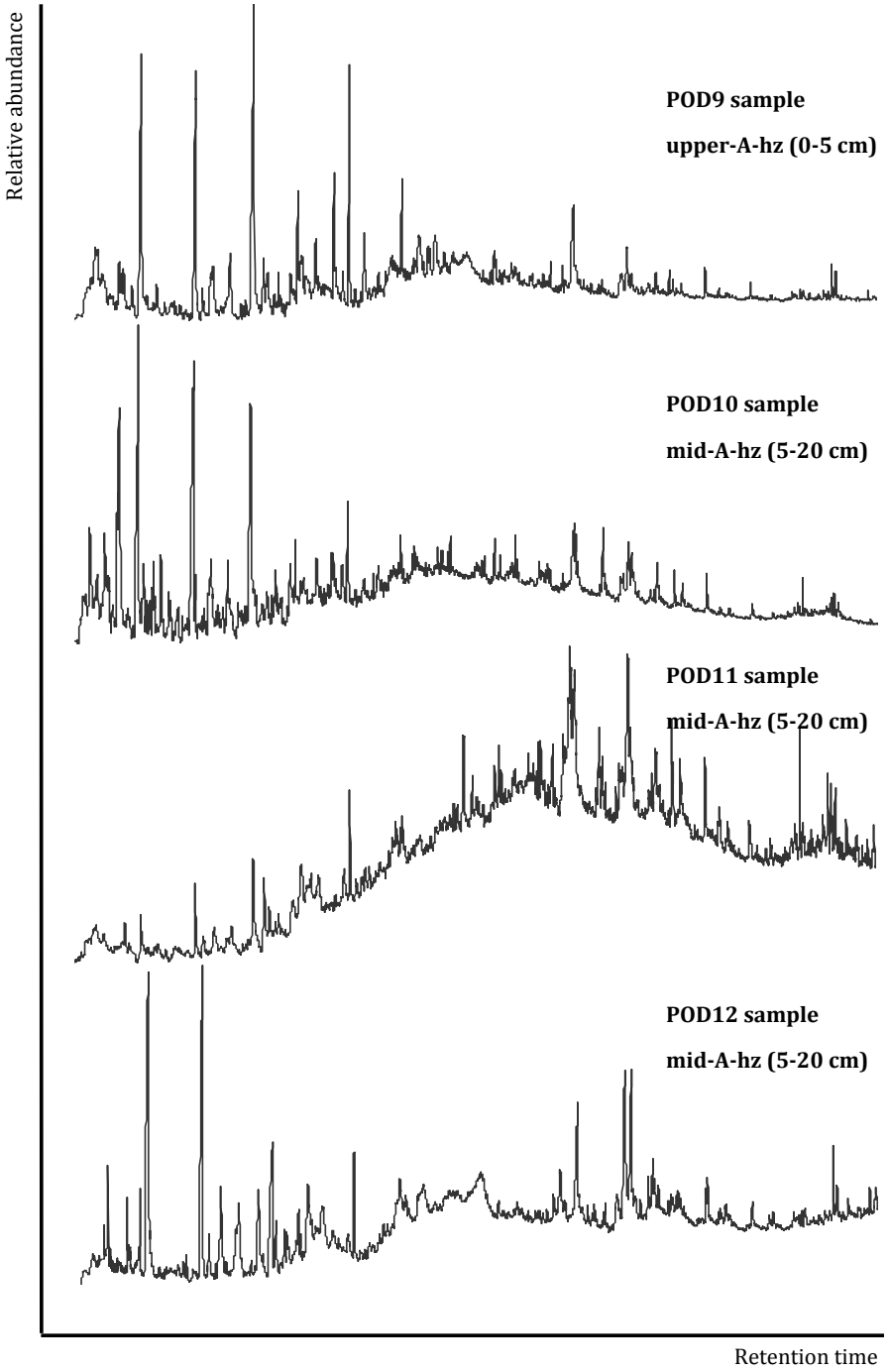
Appendix 1.2. Pyrograms of vegetal samples. *Eucalytus globulus* (analysed in chapter 3) and *Fagus sylvatica L., Pinus radiata* D. Don and *Quercus robur L*. (analysed in chapter 2).

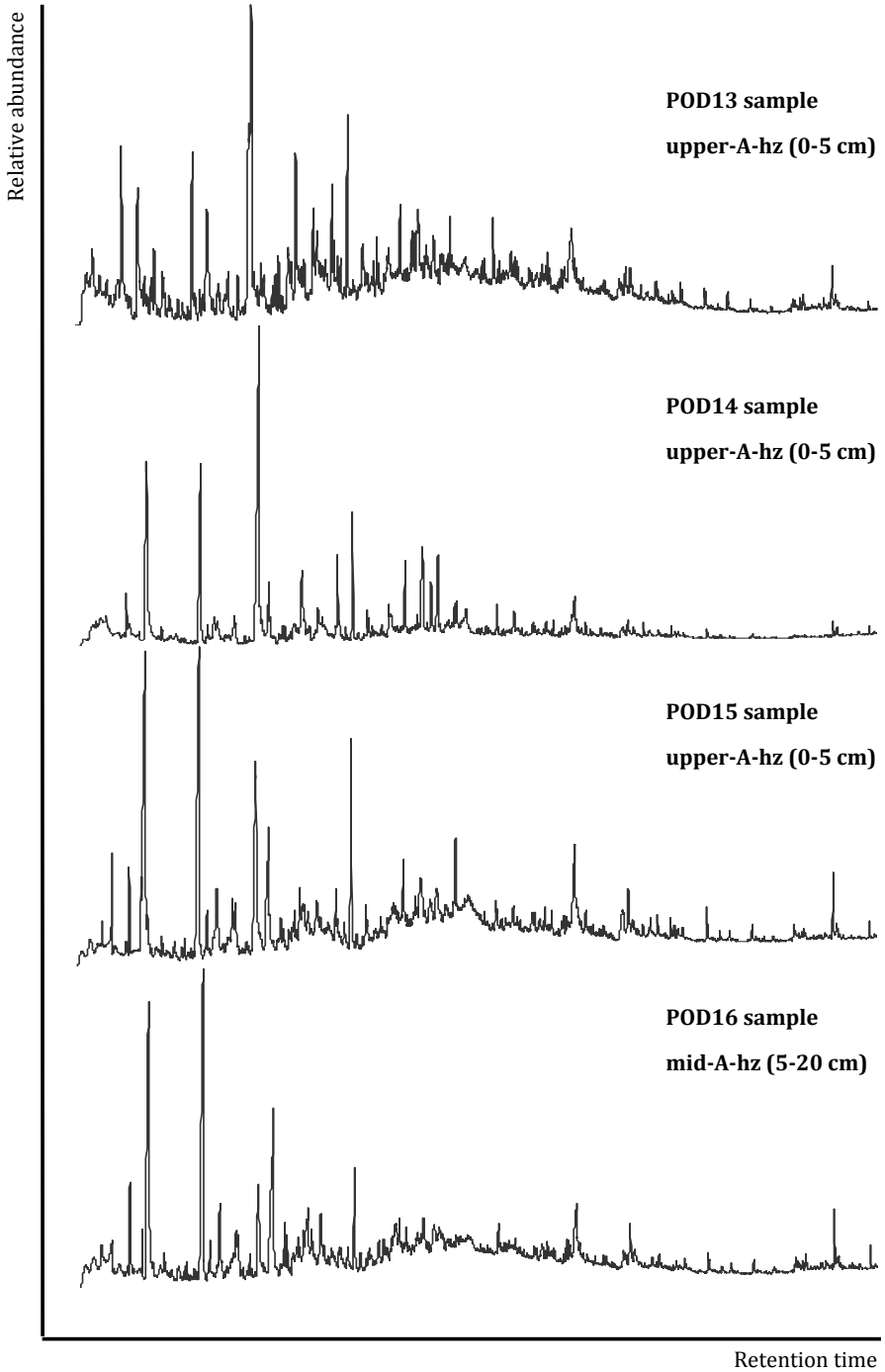


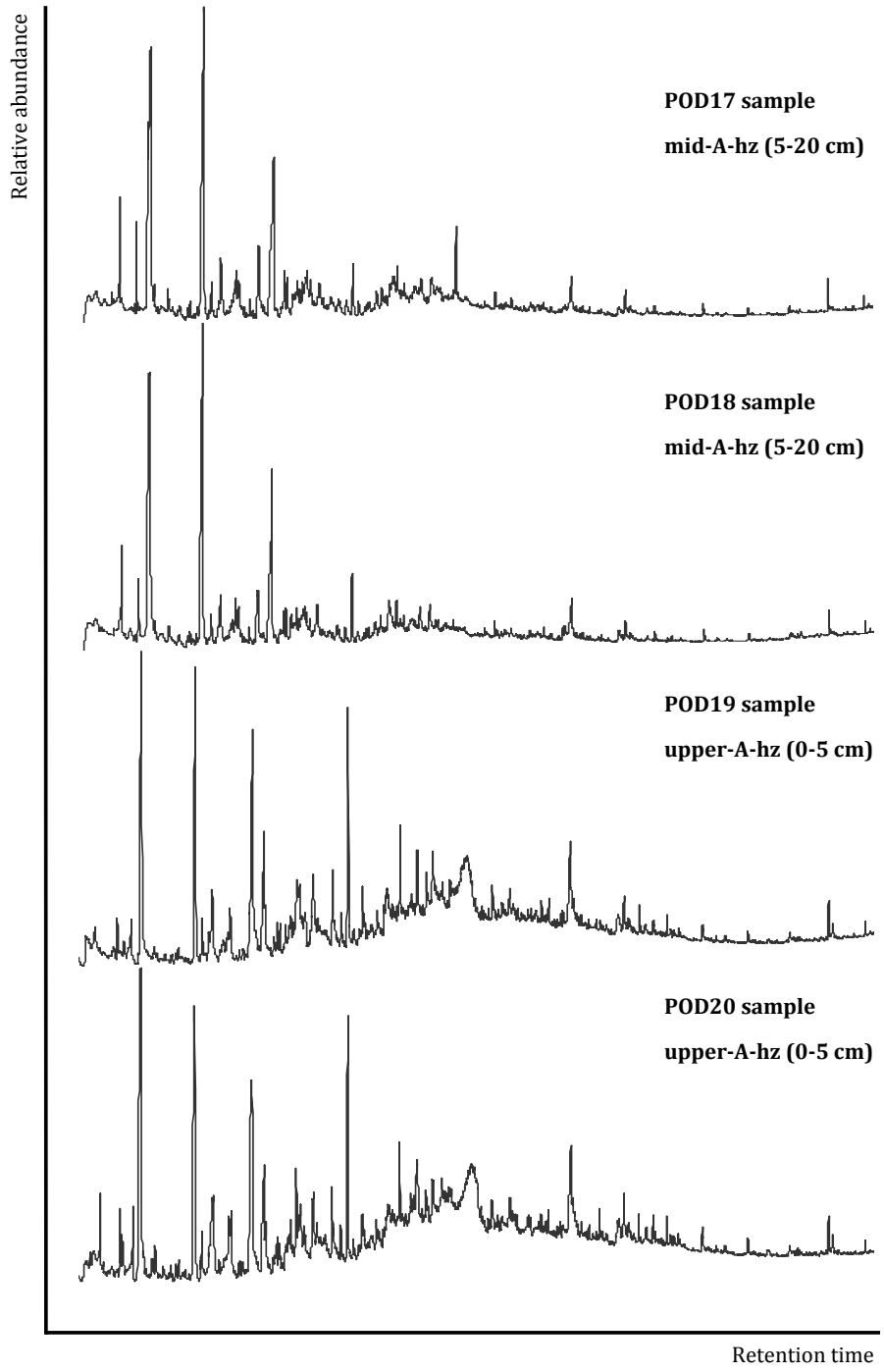
Appendix 1.3. Pyrograms of NaOH-extracted SOM of POD samples (Haplic Regosols; WRB, 2006) collected on a quartzitic massif. Moint Oiz, Basque Country (chapter 2).

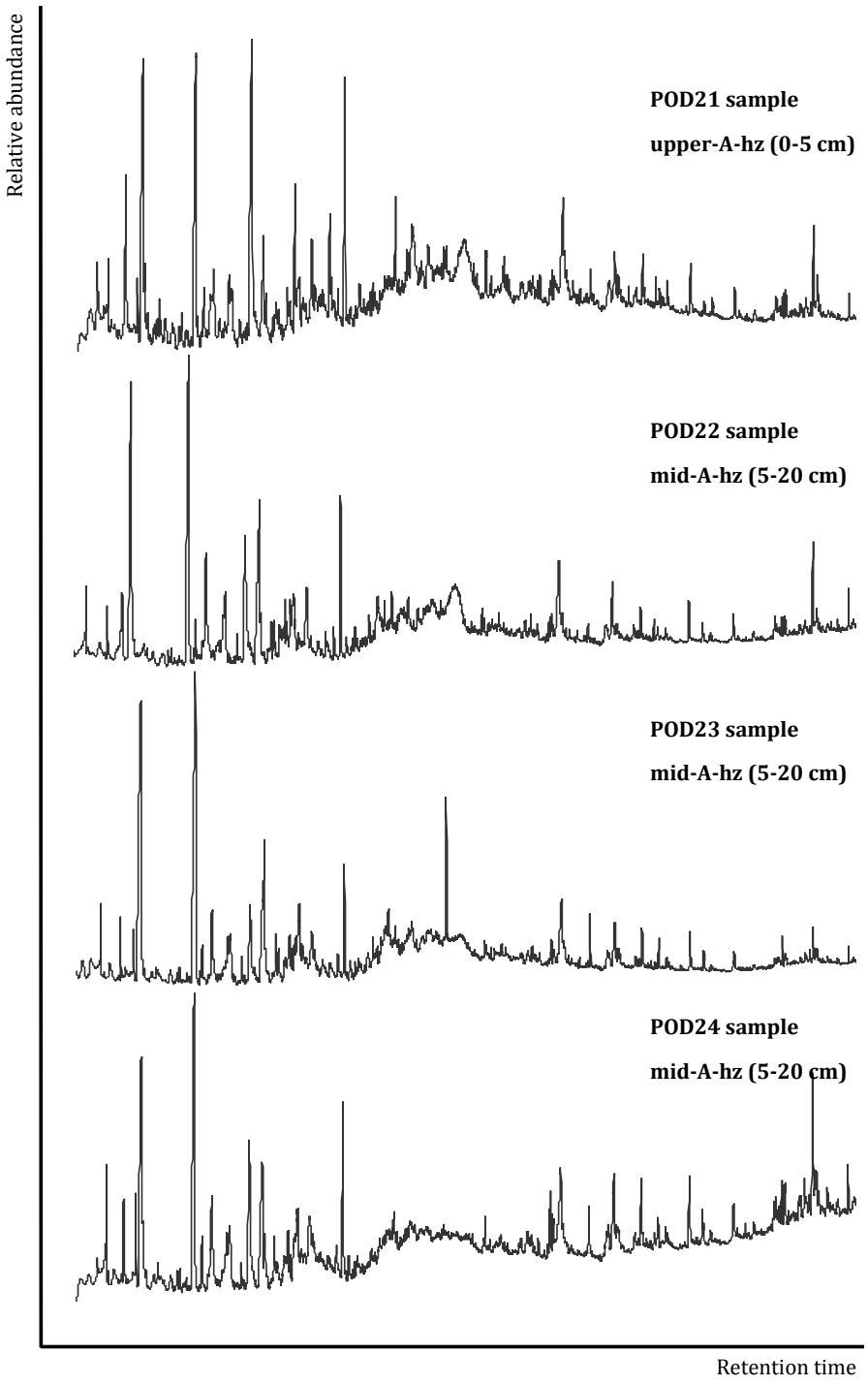



Relative abundance
POD5 sample
mid-A-hz (5-20 cm)
POD6 sample
mid-A-hz (5-20 cm)
POD7 sample
upper-A-hz (0-5 cm)
POD8 sample
upper-A-hz (0-5 cm)
Retention time

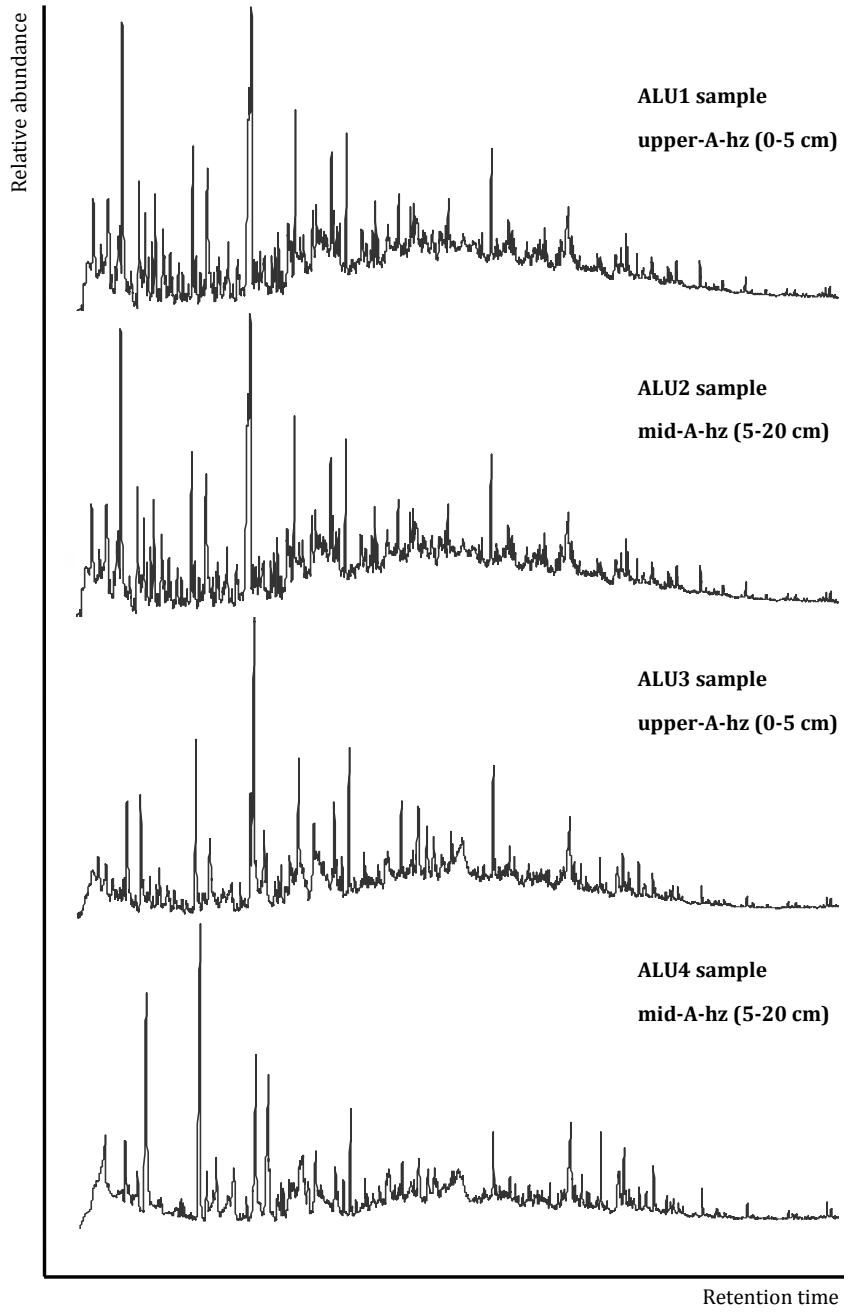

Relative abundance
POD9 sample
upper-A-hz (0-5 cm)
POD10 sample
mid-A-hz (5-20 cm)
POD11 sample
mid-A-hz (5-20 cm)
POD12 sample
mid-A-hz (5-20 cm)
Retention time

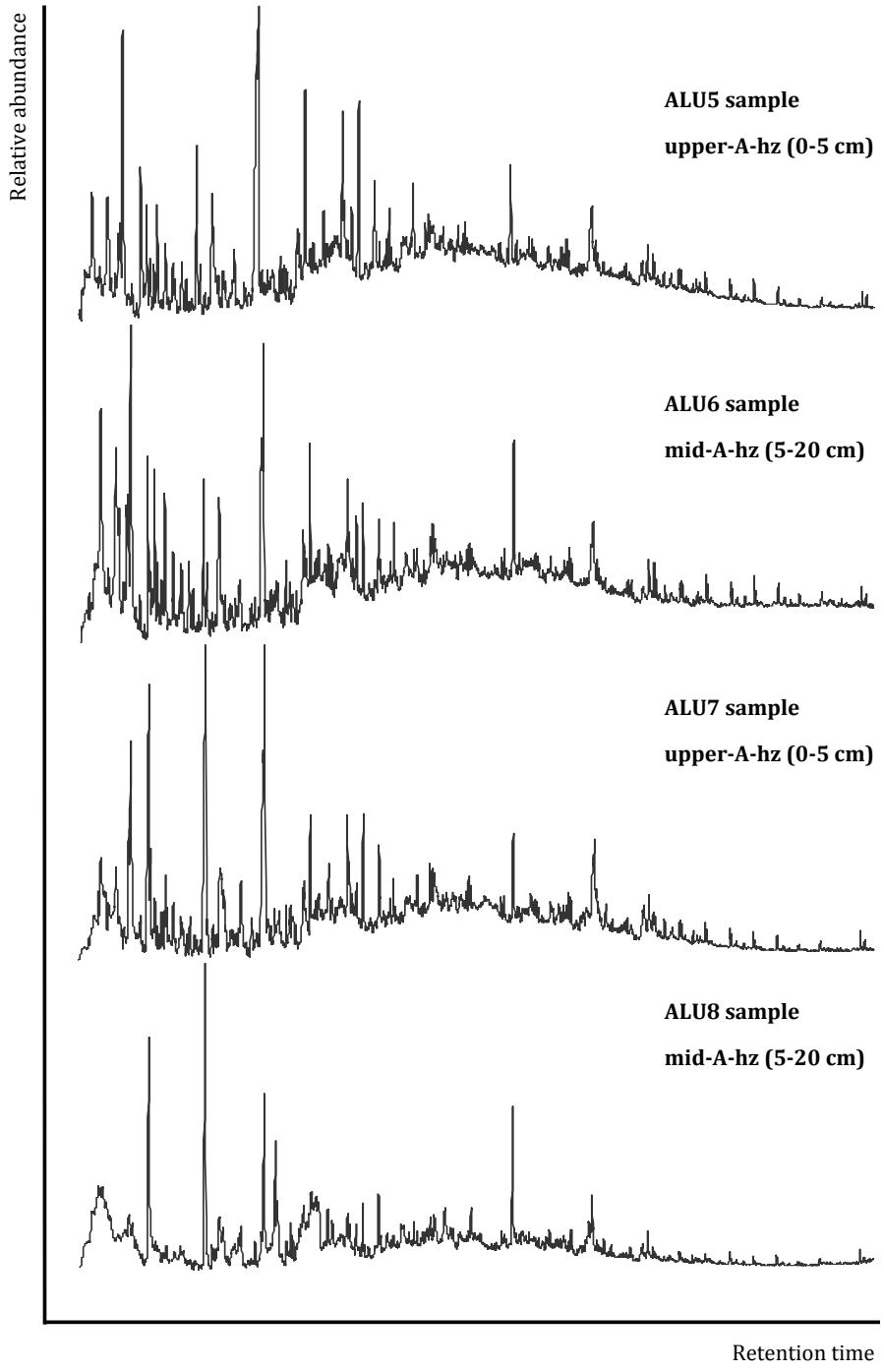

Relative abundance
POD13 sample
upper-A-hz (0-5 cm)
POD14 sample
upper-A-hz (0-5 cm)
POD15 sample
upper-A-hz (0-5 cm)
POD16 sample
mid-A-hz (5-20 cm)
Retention time

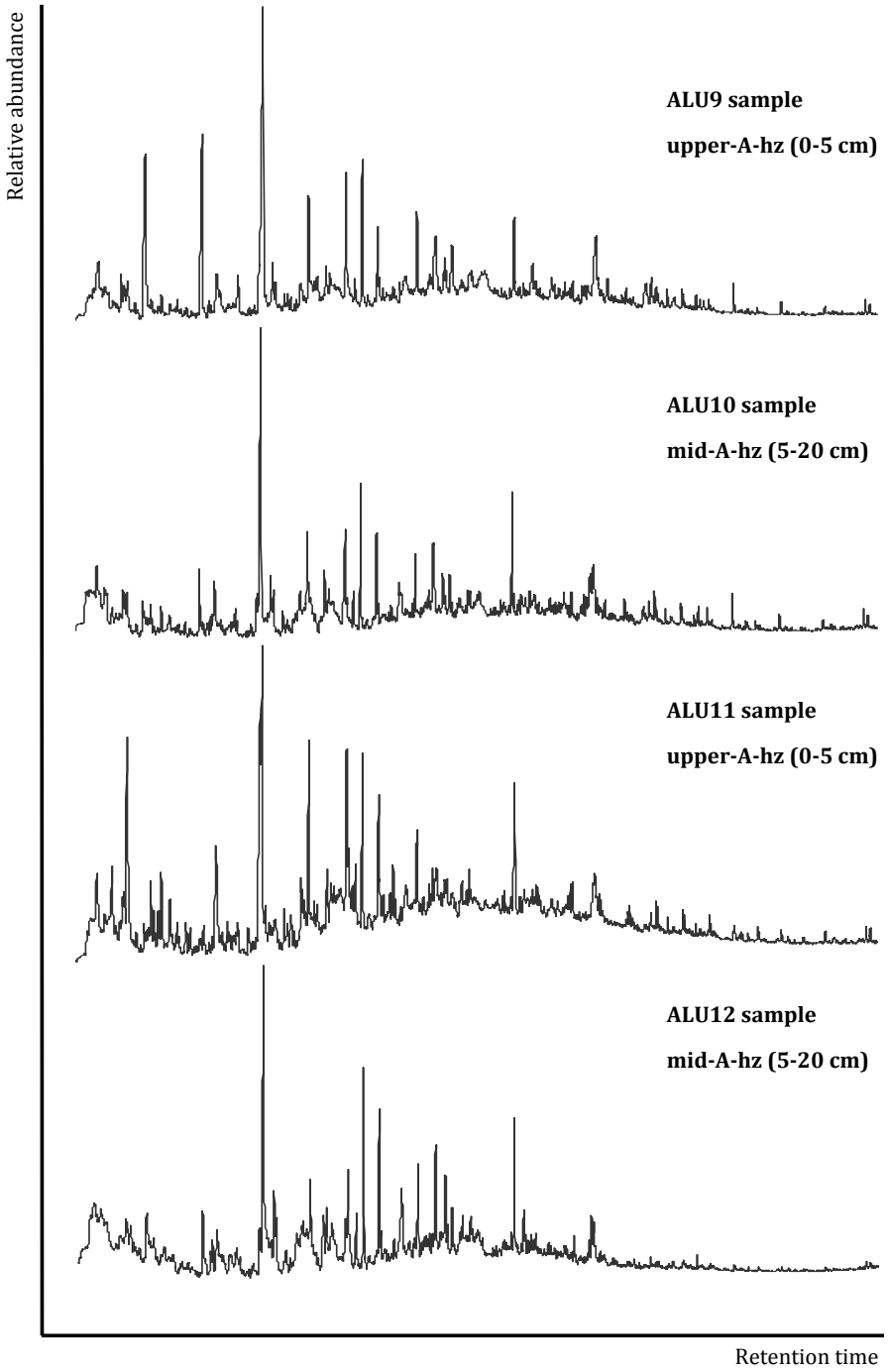

Relative abundance
POD17 sample
mid-A-hz (5-20 cm)
POD18 sample
mid-A-hz (5-20 cm)
POD19 sample
upper-A-hz (0-5 cm)
POD20 sample
upper-A-hz (0-5 cm)
Retention time

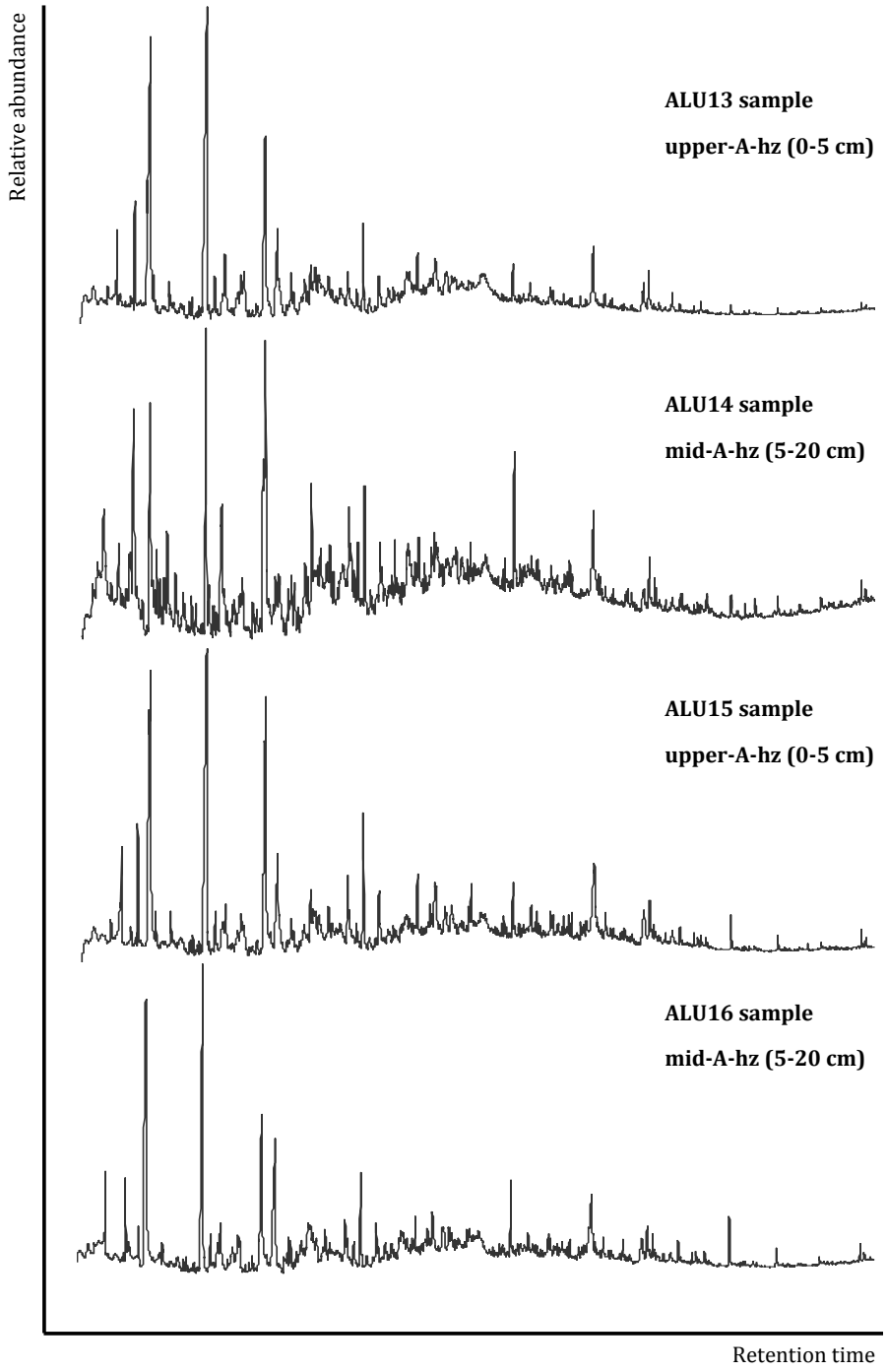

Relative abundance
POD21 sample
upper-A-hz (0-5 cm)
POD22 sample
mid-A-hz (5-20 cm)
POD23 sample
mid-A-hz (5-20 cm)
POD24 sample
mid-A-hz (5-20 cm)
Retention time

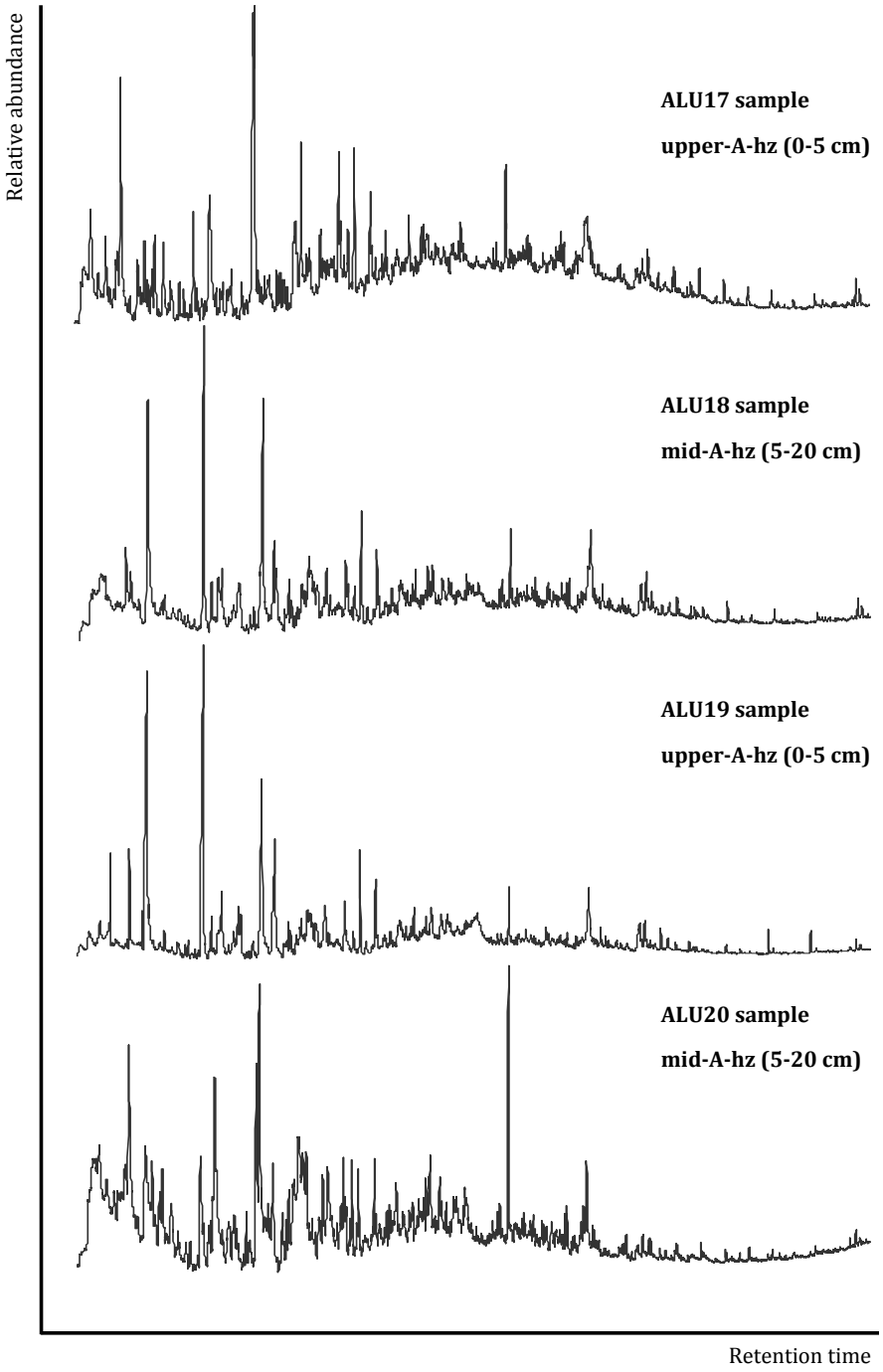
Appendix 1.4. Pyrograms of NaOH-extracted SOM of ALU samples (Cambic Umbrisols, and Umbric Leptosol) collected on a volcanic massif. Moint Karakate, Basque Country (chapter 2).



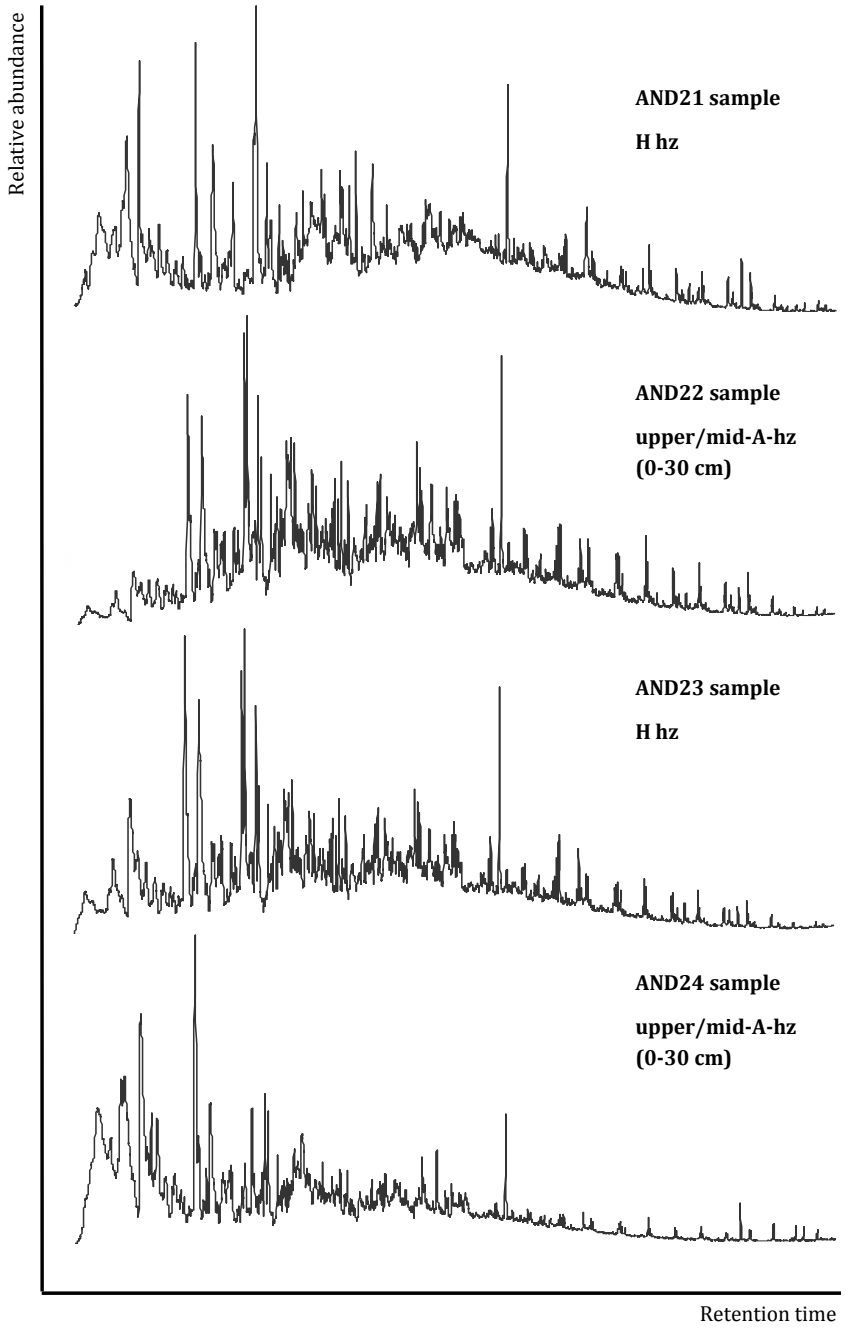

Relative abundance
ALU5 sample
upper-A-hz (0-5 cm)
ALU6 sample
mid-A-hz (5-20 cm)
ALU7 sample
upper-A-hz (0-5 cm)
ALU8 sample
mid-A-hz (5-20 cm)
Retention time

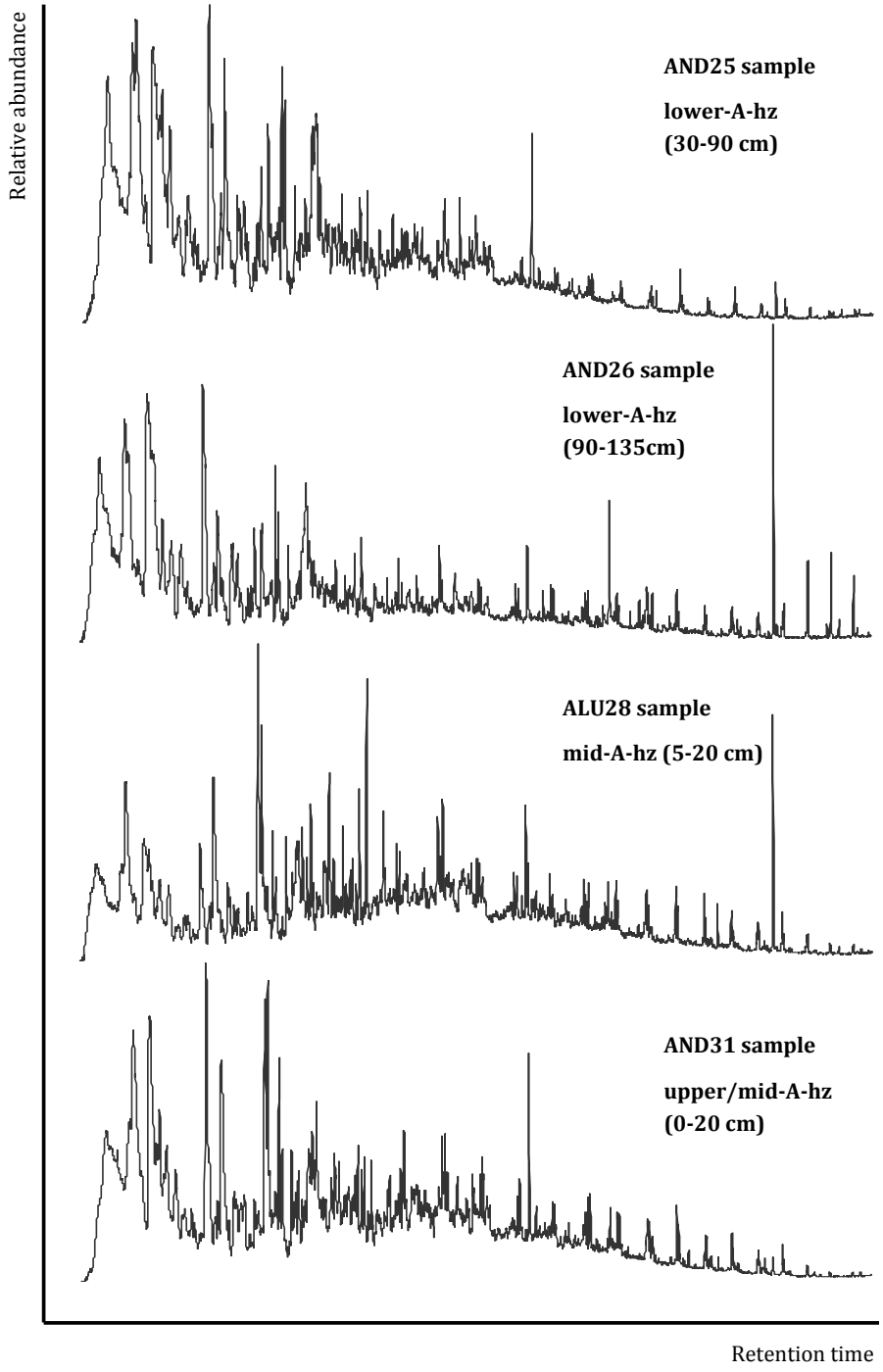

Relative abundance
ALU9 sample
upper-A-hz (0-5 cm)
ALU10 sample
mid-A-hz (5-20 cm)
ALU11 sample
upper-A-hz (0-5 cm)
ALU12 sample
mid-A-hz (5-20 cm)
Retention time

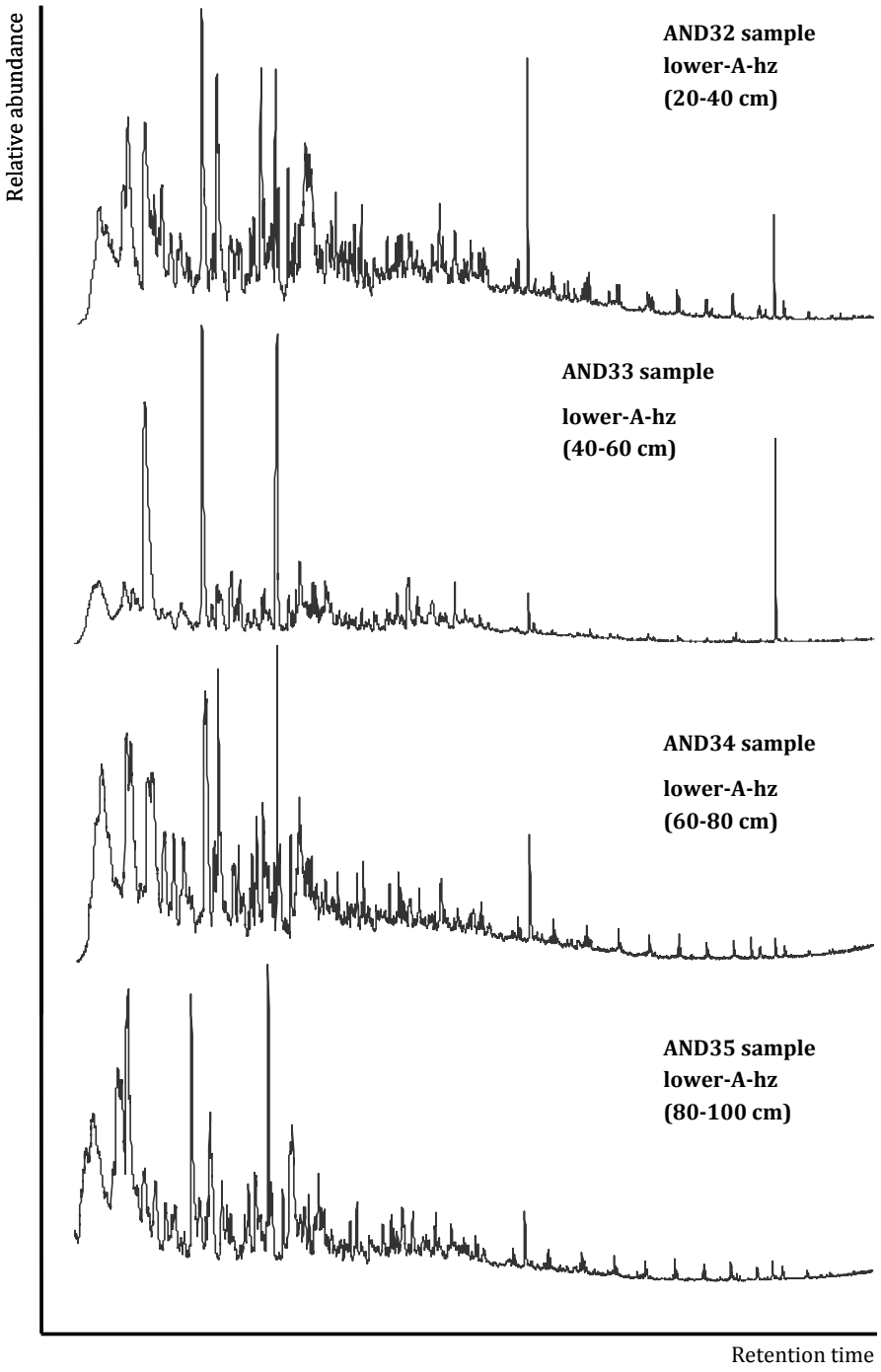

Relative abundance
ALU13 sample
upper-A-hz (0-5 cm)
ALU14 sample
mid-A-hz (5-20 cm)
ALU15 sample
upper-A-hz (0-5 cm)
ALU16 sample
mid-A-hz (5-20 cm)
Retention time


Relative abundance
ALU17 sample
upper-A-hz (0-5 cm)
ALU18 sample
mid-A-hz (5-20 cm)
ALU19 sample
upper-A-hz (0-5 cm)
ALU20 sample
mid-A-hz (5-20 cm)
Retention time

Appendix 1.5. Pyrograms of NaOH-extracted SOM of AND samples (Alu-andic Andosols; WRB, 2006) collected in a volcanic massif. Moint Karakate, Basque Country (chapter 2).




Relative abundance
AND25 sample
lower-A-hz
(30-90 cm)
AND26 sample
lower-A-hz
(90-135cm)
ALU28 sample
mid-A-hz (5-20 cm)
AND31 sample
upper/mid-A-hz
(0-20 cm)
Retention time


Relative abundance
AND32 sample
lower-A-hz
(20-40 cm)
AND33 sample
lower-A-hz
(40-60 cm)
AND34 sample
lower-A-hz
(60-80 cm)
AND35 sample
lower-A-hz
(80-100 cm)
Retention time

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